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Purification of a Vitamin by Micro-evaporation Preceding
Crystallization

What are the Vitamins

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PREFACE

WHAT are Vitamins? What do they do? Which ones do I need? How much of each do I need? How can I determine my own particular need? Where and how can I get them?

These questions are going to be on the lips of an amazingly large number of people from now on, for the public is today more than ever awake to the importance of vitamins.

How should these queries be answered?

The present text is the author's personal expression of what seems to him pertinent and reliable information—an expression resulting from his own work in the field of vitamin investigation and from his personal review of the vitamin literature.

In preparing the manuscript he is deeply appreciative of the help of his colleagues, who have read and criticized it and have tried to keep him from errors of statement and omissions of significant data. He takes pleasure in acknowledging in particular the cooperation of Drs. Thomas T. Mackie, G. N. Dalldorf and N. Jolliffe, but he fully absolves them from any responsibility for failure to cover all the pertinent data in the literature.

The story of the vitamins is today a long one, the literature enormous in volume. The present text is simply the author's personal effort to condense it without sacrifice of accuracy.

WALTER H. EDDY

New York City
December, 1940

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CHAPTER ONE

WHAT ARE THE VITAMINS?

Origin of the Name

VITAMIN means life. Although it had been known for a number of years that a mere change of diet was sufficient to cure certain kinds of diseases, the story of vitamins actually begins in 1911, when a Polish chemist, Casimir Funk, extracted from rice polishings a crystalline substance which was found to be capable of curing an Oriental disease known as beriberi. Analysis of these crystals revealed the presence of nitrogen in basic combination—that is, the so-called “amine” nitrogen. Funk therefore elected to call his extracted substance “vita-amine” or “vitamine”, the root “vita” indicating that the substance is essential to life and health. In this way the word “vitamine” was born; and you will note that it had a terminal “e”.

Four years before Funk's discovery a series of studies had been begun at the University of Wisconsin under the direction of E. B. Hart, the purpose of which was to determine the value of cereals such as wheat, corn, and oats as a cattle diet. Dr. E. V. McCollum participated in this work. Eventually he found it necessary to resort to rats to solve the

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problem of cereal differences. He transferred his studies to Johns Hopkins University, and in collaboration with Davis (1913) announced the discovery of a hitherto unknown growth factor dissolved in butter and egg-yolk fat. That this factor was not identical with Funk's "vitamine" was proved by demonstration that it did not contain nitrogen. McCollum therefore devised a new nomenclature and called his discovery "unidentified dietary factor, fat-soluble A."

McCollum's discovery was confirmed by Osborne and Mendel (1913), who had earlier found (1908-11) in milk a water-soluble growth factor for which McCollum and Kennedy later (1916) suggested the name "water-soluble B". As early as 1906 Sir Gowland Hopkins had postulated the existence of growth factors effective in far too minute amounts to be classed as foods or nutrients. For them he suggested the name "accessory factors"; but this name was unsatisfactory since further study proved that these products were actually essential to health and nutrition. Between 1911 and 1920 three of these disease-preventing and growth factors were definitely demonstrated: the anti-beriberi factor water-soluble B, the anti-eye disease factor fat-soluble A, and the anti-scurvy factor water-soluble C.

The phraseology "unidentified dietary factors fat- or water-soluble X" was extremely cumbersome. On the other hand Funk's "amine" suffix didn't apply to A or C, for neither contained any nitrogen. Dr. J. C. Drummond therefore proposed (1920) a simplification of nomenclature, consisting of dropping the final "e" from Funk's "vitamine" and combining this with McCollum's alphabetical designations, *viz.*, unidentified dietary factors are to be called "vitamins" and distinguished by the letters A, B, C, etc. By this proposal

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McCollum's "unidentified dietary factor, fat-soluble A" condensed to vitamin A. This suggestion was generally adopted and explains the present letter usage.

Vitamins Are Organic Chemical Compounds

The discoveries reported above proved that food contains substances which in very small amounts are capable of affecting growth and preventing certain types of disease. Funk's success (1911) in isolating from rice polishings a crystalline product which cured beriberi, and his analysis of these crystals indicated that these substances are organic chemical compounds; and Funk's name of "vita-amine" indicated his belief that they were compounds containing basic nitrogen, *i.e.*, NH_2 groups.

Today the successful isolation and synthesis of ten of the substances has proved that part of Funk's hypothesis was correct, and that vitamins are organic chemical compounds. They have also shown, however, that the different vitamins have little in common so far as chemical structure is concerned. Some proved to be sugar acids, some sterols, some nitrogen-containing compounds and some without any nitrogen at all. Funk's vita-amine, then, is not a satisfactory name for the group because so far, those containing no amine (NH_2) group are the more numerous. And even the modified term "vitamin" today merely indicates that these substances are of similar physiological significance rather than of similar chemical character. This fact is made clearer by the description of the chemical nature of those vitamins already chemically identified, which will be found in Appendix A.

We may then define a vitamin as a chemical compound

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whose presence in diet is essential to the maintenance of growth and health, and whose absence from the diet or inadequate supply results in the development of specific manifestations of ill health or pathology.

Vitamins differ from what we call essential nutrients in several particulars. First, the amounts essential are far smaller than of ordinary nutrients such as proteins, fats, carbohydrates and minerals. Secondly, they are more or less changeable and may be "inactivated" by temperature, oxidation, etc. Thirdly, they may exist in inactive form and require special treatment, such as irradiation or enzyme action, to become physiologically efficient. Vitamins occurring in inactive form in nature are called provitamins. Carotene, for example, is provitamin A, and ergosterol is provitamin D₂.

Kinds of Vitamins

Because some vitamins are soluble only in fats or fat solvents and others soluble in water, their separation into these two groups is common. The earliest to be discovered were designated by letter; but some of the later ones have received descriptive names, and still others have been renamed as their chemical identity has been revealed and a chemically descriptive designation made possible.

In the following table those definitely identified and those postulated but not yet isolated, with their various synonyms, are given to show the present status of the group. There is no reason to assume that this list is complete, and it is possible that some of the postulated ones may later prove to be duplicates of some already isolated. For example, Carter and O'Brien (1939) claim that what were originally classified

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Table 1.

A. Vitamins Chemically Identified:

Designation	Chemical Name	Function
Vitamins A ₁ and A ₂	Activated Carotene	Antixerophthalmic
Vitamin B ₁	Thiamine or Aneurin	Antineuritic
Vitamin B ₂ or G	Riboflavin	Anti-rat-dermatitis
Vitamin B ₆	Pyridoxine	Anti-acrodynia
Vitamin-P-P	Nicotinic Acid or Amide	Anti-pellagra
Vitamin C	Ascorbic Acid	Anti-scorbutic
Vitamin D ₂	Calciferol	Anti-rachitic
Vitamin D ₃	7-dehydro-cholesterol	Anti-rachitic
Filtrate Factor	Pantothenic Acid	Anti-chick-dermatitis
Vitamin E	Tocopherol	Anti-sterility
Vitamin K	Substituted 1,4-naphthoquinones	Coagulation
Vitamin P	Eriodictyol—Szent Gyorgyi's	Capillary permeability control factor

N.B. For description of chemical nature of these vitamins see Appendix.

B. Vitamins Postulated on Physiological Evidence but not yet Isolated or Identified Chemically:

Vitamin B ₃	William's and Waterman's bird-weight maintenance factor (possibly pantothenic acid).
Vitamin B ₄	Reader's rat paralysis preventive factor.
Vitamin B ₅	Peters' heat-stable bird-weight maintenance factor (possible pyridoxine).
Vitamin H	Parson's and Gyorgyi's anti-egg-white dermatitis preventive factor (Biotin, Coenzyme R).
Vitamin I	Centanni's digestive factor.
Vitamin J	Von Euler's anti-guinea-pig-pneumonia factor.
Vitamins L ₁ and L ₂	Nakahara's lactation control factors.
Vitamin M	Day's anti-monkey pellagra factor.
Vitamin U	Stokstadt-Manning chick-growth factor.
Vitamin W and B _w	Rat growth filtrate factor.
Grass juice factor	
Anti-grey hair factor	(Possibly identical with Pantothenic Acid)
Spectacled eye factor	
Adrenal necrosis factor.	
Cartilage factor	

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as vitamins B₃ and B₅ are probably what we now call pantothenic acid and pyridoxine respectively.

In this list is included only those factors demonstrated to be concerned with animal physiology. Human need for some of them has not yet been demonstrated. There are other substances similar in behavior toward the growth and development of plant life, but it is generally agreed that the term "vitamin" be restricted to those which have been proved to affect animal behavior.

The group of physiological compounds to which the vitamins seem most comparable is the hormone group (secretions of the endocrine glands) and vitamins are sometimes called "food hormones" to distinguish them from hormones of internal secretion, such as insulin, thyroxin, etc.

Relation to Enzymes

Study of biological oxidation has shown that many of the vitamins are actively concerned in oxidation reactions. They form important action, or "prosthetic" groups in the enzymes and coenzymes that the body uses for transport of hydrogen or activation of oxygen in the oxidations by which food energy is made available (See Chapter Two).

Availability and Potency

Vitamins are distributed in nature in natural foodstuffs. Before they were isolated, their existence was determined by the biologic response of test animals such as the rat, guinea pig, dog and chick. The kinds and amounts vary with the foodstuff, some foods being rich in certain vitamins and low

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or lacking in others. Because their presence and amount was determined to a large degree by bioassays, quantity was originally expressed in terms of animal response. For example, Sherman (1925) first defined a unit of vitamin A as the amount of vitamin A source necessary, in a diet complete in all other factors, to produce a gain of 3 grams weekly in rats, provided that the rats had been depleted of their stored vitamin A before the test began. The original unit of vitamin C was the least amount of vitamin C source that would prevent development of scurvy in a guinea pig; a unit of vitamin D the least amount needed to secure what was called two-plus healing in the bones of a rat having rickets.

These methods of expressing vitamin potency led to considerable confusion as different assayists defined their own units. For instance, at one time vitamin D potency was expressed in Steenbock Units, Poulsson Units, A.D.M.A. units, and rat units.

To eliminate this confusion the Nutrition Section of the League of Nations appointed a Committee to set up what are now known as International units. Fortunately, the availability today of certain of the vitamins in pure form has made it possible to translate these definitions into actual weight of specific vitamin substance. In Table 2 is given the present definition of unitage of those for which we have a test method; in Table 3 equivalents now in use.

Therapeutic Sources of Vitamins

In the table in Appendix B will be found the approximate vitamin distribution in common foodstuffs. In addition, today, vitamin preparations containing vitamins A, B₁, B₂, B₆,

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Table 2. Vitamin Potency.

Vitamin	Unit Definition	Available as Reference Material
A	The amount of source capable of producing the physiological effect of .0006 mg. pure beta-carotene equals one International unit.	U.S.P. Reference Cod Liver Oil containing 1700 International units per gram.
B ₁	The amount of source capable of producing the physiological effect of .003 mg. pure thiamine equals one International unit.	Crystalline thiamine
B ₂ (G)	The amount of source capable of producing the physiological effect of .0025 mg. pure riboflavin is one Sherman-Bourquin unit.	Pure riboflavin
B ₆	The amount of source capable of curing acrodynia in 3 weeks is the Schneider, Ascham, Platz, Steenbock (1939) unit. It represents approximately 0.1 mg. B ₆ crystals.	Pure pyridoxine
C	The amount of source capable of producing the physiological effect of .05 mg. pure l-ascorbic acid equals one International unit.	Pure l-ascorbic acid
D	The amount of source capable of producing the antirachitic effect of .000025 mg. pure calciferol equals one International unit.	Pure calciferol and U.S.P. Reference Oil containing 115 International Units of D per gram.
E	The amount of source capable in 21 days of rat gestation to insure birth of a litter is an Evans (1922) unit.	Standardized wheat-germ oil
K	The amount of source required per gram of animal weight on three successive days to bring chick blood clotting time to normal is a Dam (1935) unit.	Standardized alfalfa meal extract and synthetic K ₁ .

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Table 3. Vitamin Unit Equivalents.

N.B. The U. S. Food and Drug Administration now requires that vitamins A, B₁, C and D potencies be expressed on labels in International units and B₂(G) potency in micrograms of riboflavin.

Vitamin A

- 1 International unit is equivalent to .0006 mg. beta-carotene = 1 U.S.P. unit
- 1 International unit is equivalent to 0.7 Sherman unit
- 1 gram U.S.P. Cod liver oil must contain at least 850 International units of A per gram

Vitamin B₁

- .003 mg. pure thiamine
- 2 Sherman-Chase units
- 0.5 Smith curative unit
- 1.0 Chick-Roscoe unit
- 2.0 Cowgill mg. equivalent units

Vitamin C

- 1 International unit is equivalent to .05 mg. l-ascorbic acid
- 1 International unit is equivalent to 0.1 Sherman-La Mer unit
- 1 cc. average orange juice contains approximately 8.5 International units

Vitamin D

- 1 International unit is equivalent to .000025 mg. calciferol = 1 U.S.P. unit
- 1 International unit is equivalent to 3.25 A.D.M.A. units
- 1 International unit is equivalent to 0.37 Steenbock unit
- U.S.P. Cod liver oil must contain at least 85 International units per gram

Viosterol must contain at least 10000 International units per gram
A product labelled 250 D or 150 D etc. indicates 250 or 150 times the D content of a potent cod liver oil used as standard. According to Council of Pharmacy a Viosterol labelled 250 D contains 3333 A.D.M.A. rat units per gram or approximately 10000 International units per gram.

Vitamin B₂ or G

- 1 Sherman-Bourquin unit is equivalent to 2.5 micrograms of riboflavin

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The Vacuum Distillation of an Intermediate in the Synthesis of
a Vitamin

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nicotinic acid, D, and E, singly or in combinations, are now available to the physician in capsule or tablet form, and there are also standardized high-potency fish-liver oils as sources of vitamins A and D. Labelling laws compel the proper indication of potency in units on such preparations and therefore permit quite a wide range of choice for therapeutic purposes.

In that connection it is important to bear in mind the probable fact that requirement of one vitamin may be conditioned by adequacy or inadequacy in others and that we shall need much study of vitamin combinations before we can definitely determine optimum requirements of any one type. Each pure form the drug laboratories supply provides a tool for contrast of extracts of natural foods with the pure types to move further toward the goal of adequate vitamin therapy.

Bibliography

- Carter, C. W., and O'Brien, J. R., Proc. 7th World's Poultry Congress, Cleveland, p. 126, 1939. Probable Nature of Vitamins B₃ and B₆.
- Dam, H., *Biochem. J.*, 29, 1273 (1935). The Antihemorrhagic Vitamin of the Chick.
- Drummond, J. C., *Biochem. J.*, 14, 660 (1920). The Nomenclature of the So-Called Accessory Food Factors.
- Evans, H. M., *Am. J. Physiol.*, 63, 396 (1922). On the Existence of a Little Known Dietary Factor Essential for Reproduction.
- Funk, C., *J. Physiol.*, 43, 395 (1911). On the Chemical Nature of the Substances Which Cure Polyneuritis in Birds Induced by a Diet of Polished Rice.
- Hart, E. B., McCollum, E. V., Steenbock, H., and Humphrey, G. C. Wis. Exp. Sta. Bull. No. 17, 1911. Calf Experiments.
- Hopkins, F. G., *The Analyst*, 31, 385 (1906). The Analyst and the Medical Man.
- McCollum, E. V., and Davis, M. *J. Biol. Chem.*, 15, 167 (1913). The Necessity of Certain Lipins in the Diet. *J. Biol. Chem.*, 19, 245 (1914). Observation

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on the Isolation of the Substance in Butter Fat Which Exerts a Stimulating Action on Growth.

McCollum, E. V., and Kennedy, C., *J. Biol. Chem.*, 24, 491 (1916). Water-Soluble B.

Osborne, T. B., and Mendel, L. B., Carnegie Yearbook, 1911. Protein-free Milk Factor. *J. Biol. Chem.*, 16, 423 (1913). Influence of Natural Fats on Growth.

Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., *J. Nutrition*, 18, 99 (1939). The Anti-acrodynic Properties of Certain Foods.

Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 47, 1639 (1925). The Quantitative Determination of Vitamin A.

CHAPTER TWO

WHAT DO THE VITAMINS DO?

Vitamins and Enzymes

ONE of the outstanding discoveries of recent years has been that certain vitamins function as prosthetic or action groups in the enzyme systems that control cell respiration and intracellular metabolism. Tests prior to these discoveries showed that vitamin deficiency could reduce growth rate and produce definite pathological conditions in the deprived animals. We had learned that to prevent scurvy, rickets, beriberi, pellagra, etc., we must supply certain specific vitamins in adequate amounts. But we lacked evidence as to how the vitamins accomplished this protective action. We still lack complete data on the way in which vitamins act, but the relation of some of them to intracellular respiratory enzymes clears up some of the problems.

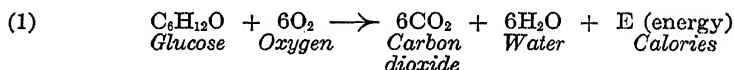
Before discussing the functions of individual vitamins, therefore, it is perhaps worth while to devote a little space to how respiratory enzymes operate and how the presence in these enzymes of certain vitamins as structural elements controls their performance.

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What is Cell Respiration?

Respiration, or breathing, is generally recognized as the process of taking in oxygen and giving out carbon dioxide. The carbon dioxide is produced by the oxidation of food-stuffs in the body tissues to which the oxygen is brought by the blood, and water as well as carbon dioxide is produced in the cells by the oxidation process. Explanation of intracellular respiration, then, requires information as to what substances are oxidized and how they are converted into carbon dioxide and water.

Physiologists have known for many years that the principal fuel food is sugar; that when sugar is oxidized in tissue cells it is converted into water and carbon dioxide; and that energy is liberated by this process. They have expressed this reaction as follows:



They have also known that when two hydrogen atoms unite with one oxygen atom 68 calories of energy are the maximum calories obtainable.

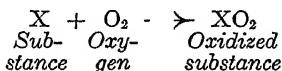
But this simple equation fails to show all the steps between the arrival of sugar in a cell and the production of water and carbon dioxide, or the compounds used in producing this result. Furthermore we now know that oxidation may be accomplished in several ways. What are these ways?

Methods of Oxidation

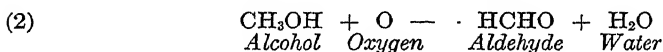
The simplest form of oxidation is a chemical union of oxygen with the substance oxidized. When iron rusts, coal

WHAT DO THE VITAMINS DO?

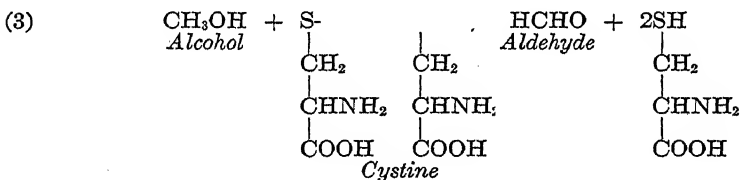
burns, metals corrode, or fats turn rancid we have examples of such unions that may be expressed as follows:



Another form of oxidation takes place when, instead of uniting with the substance oxidized, the oxygen removes hydrogen from it. The conversion of alcohol to aldehyde is an example of such an oxidation, *e.g.*,

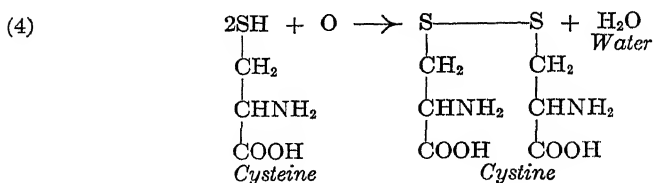


In such an operation we say that oxygen acts as a hydrogen acceptor; and if hydrogen acceptance is "oxidation," it follows that any substance that can "accept" hydrogen is an oxidizing substance. Such a substance need not be oxygen; in fact, there are other substances than oxygen which can and do accomplish oxidization in the body by hydrogen acceptance, *e.g.*, cystine, glutathione, thus:



In this case the sulfur in the cystine has operated just like oxygen in equation (2) in removing hydrogen from the alcohol and thus oxidizing it to aldehyde. This is what scientists call an anaerobic oxidation since it takes place without the presence of oxygen itself. Furthermore, if we now bring cysteine into contact with oxygen it can be converted back to cystine, thus:

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We have in equations (3) and (4) an anacrobic oxidation followed by an aerobic oxidation and it may be seen that by this double process a small amount of cystine would serve to convert alcohol continuously to aldehyde.

But there is still another form of oxidation involving removal of charged particles, or electrons, from a compound. We picture the atom today as a system composed of a central nucleus of one or more positively charged particles, called *protons*, surrounded by a usually more numerous collection of negatively charged particles, called *electrons*. The simplest atom we know is hydrogen, which consists of a single proton and a single electron. Under certain conditions this electron can be removed, leaving the atom with only the positive charge. Atoms which lose or gain electrons therefore become positive or negative radicals, or *ions*; their system has an excess positive or negative electrical charge.

On the electron basis, *valence* may be defined as the number of electrons that an atom of that element loses or takes up in entering into combination with atoms of other elements. Since an atom of hydrogen has only one electron to lose, the hydrogen ion has a valence of one, and is positively charged, H^+ . An atom of chlorine can take up only one electron and hence is also univalent; but its ion is negatively charged, Cl^- . Certain elements can exist, however, with more than one valence. Iron, for example, can lose two electrons

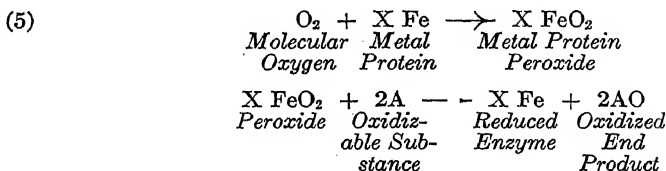
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and become the divalent ion Fe^{++} or it may lose still another electron and become the trivalent ion Fe^{+++} . Oxygen can accept two electrons and become the divalent negative ion O^{--} . Increase in valence is also called oxidation; consequently if a substance can accept or remove an electron from an element or compound it is said to be an *oxidizing* agent. Conversely, addition of an electron reduces valence and an electron donator is therefore a *reducing* agent.

All these methods of oxidation have been demonstrated to occur in living tissues and explanation of cell respiration involves explanation of how these processes happen and what makes them proceed. In summary then we may have:

- (a) Oxidation by addition of oxygen itself to a compound.
- (b) Oxidation by removal of hydrogen from a compound.
- (c) Oxidation by removal of an electron from a compound.

Warburg's (1925) view of oxygen activation was originally expressed by equation (5):



The theory was that the molecular oxygen was changed to nascent oxygen (that is, oxygen in process of formation) by formation of the peroxide X FeO_2 , and that nascent oxygen could react with the substrate (2A), whereas molecular oxygen (O_2) was inert.

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In the light of later knowledge this nascent oxygen theory is not a necessary explanation of what happens through the action of the metal-protein enzyme. We now incline to explain it as follows:

(6) First Step: Four hydrogen atoms lose an electron each, becoming positively charged hydrogen ions (H^+).

Second Step: $4 X Fe^{++}$ metal proteins accept these 4 electrons and become $4 X Fe^{+++}$ or oxidized metal protein.

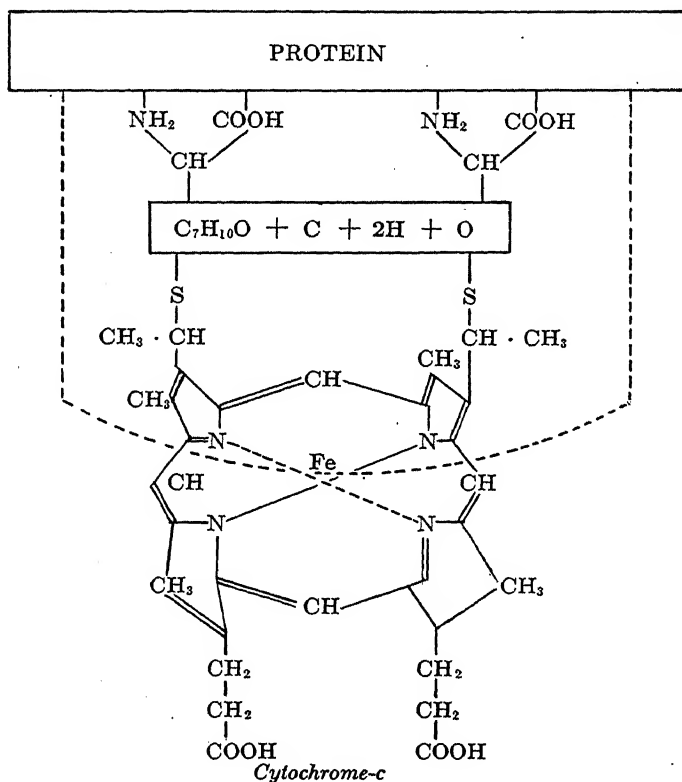
Third Step: The four molecules of oxidized metal protein ($4 X Fe^{+++}$) then pass their four electrons on to two molecules of oxygen ($2O_2$) which in turn becomes ionized ($2O^{--}$) and the metal protein goes back to the reduced form ($4X Fe^{++}$).

Fourth Step: The four hydrogen ions and the two oxygen ions then unite to form two molecules of water.

The metal protein, in other words, would have accomplished the activation of oxygen and its union with hydrogen by simply passing on the negatively charged electrons from hydrogen to oxygen; by alternately acting as an electron acceptor and electron donator, the metal protein, would become alternately oxidized and reduced. This process could go on indefinitely so long as there was hydrogen to donate electrons and oxygen to receive them. This is the modern conception of how the metal-protein-oxidases function. Compounds having the ability to pass on electrons in this fashion have been isolated and are called "cytochromes."

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Theorell's (1938) reconstruction of one of these metal proteins, or cytochromes, is shown below:



But Warburg's discovery of the need to activate oxygen was only part of the story. It was Wieland (1912) who showed that it was also necessary to activate the hydrogen in the substrate to make it "let go". He showed that this was also accomplished by enzymes which are called today *dehydrogenases*. They consist of a protein to adsorb the substrate and a prosthetic or action group capable of accept-

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ing hydrogen. The combined effect of dehydrogenase and oxidase, then, produces the following steps:

- a. Substrate plus specific dehydrogenase gives up two hydrogens.
- b. Oxygen plus specific oxidase is made an acceptor of hydrogen.
- c. Result substrate loses hydrogen which passes to the oxygen; water is formed and energy produced.

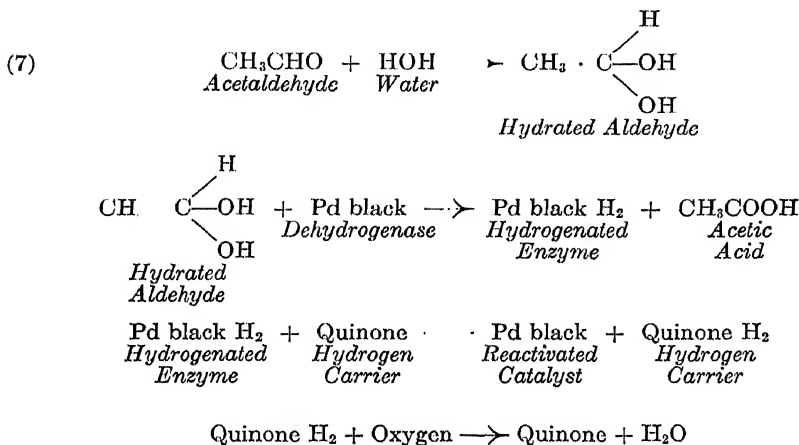
Hydrogen Carriers

But these enzymes are not the only factors involved in intracellular oxidation. It is known that two chemical compounds or elements cannot react with one another if the distance between them is greater than 0.00000045 of a millimeter. Also, as we noted at the beginning of our discussion of activation, if hydrogen unites directly with oxygen the entire 68 calories are released with explosive violence. If we could break down this release into a series of steps, such a gradual release might be highly desirable. In fact, we know it is highly desirable and necessary in living tissue cells.

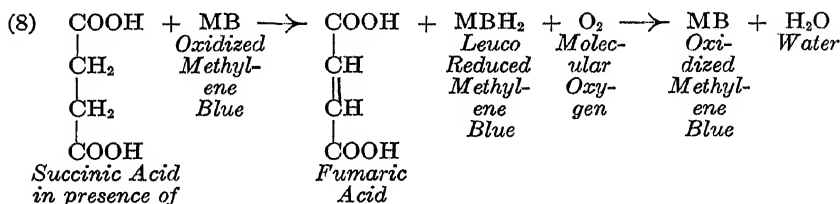
A search for the ways in which this gradual release and gap-bridging is accomplished in the cell revealed a whole series of tools used by the cell for this purpose. These tools today are collectively spoken of as hydrogen carriers because they function by taking hydrogen from the substrate, often passing them on to more than one other carrier, and finally delivering them to the oxygen.

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Wieland demonstrated this process of dehydrogenation and hydrogen carriage by an experiment in which palladium black functioned as the catalyst, or enzyme, and quinone as the hydrogen carrier, as follows:



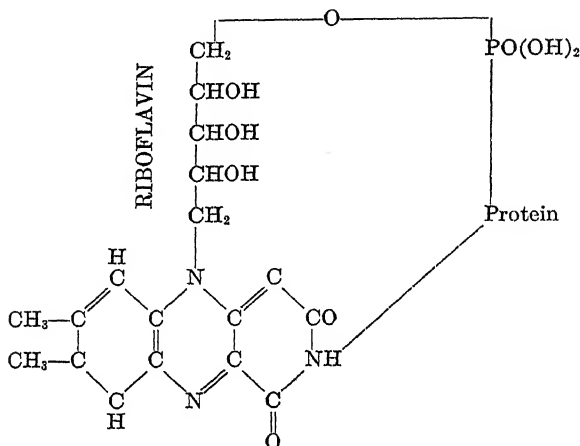
Certain pigments or dyes have been known for some time to be capable of hydrogen acceptance, for example, methylene blue. Its method of action is shown in Equation (8):



In 1932 Warburg reported the discovery of a "yellow respiration enzyme" which combined the action of dehydrogenase and hydrogen acceptor and contained no metal. This

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enzyme was shown by Theorell (1937) to have the following structure:



This compound proved of special interest to vitamin students because the riboflavin part of it proved identical with what we know today as vitamin B_2 or G. It was the first enzyme shown to contain a vitamin as a part of the prosthetic group.

Today other flavin-proteins have been isolated from oxidation enzyme systems. In other words, Warburg's yellow enzyme is not the only flavin protein that has been isolated. In 1938 Corran and Green reported recovery of a flavo-protein from milk in which the prosthetic group appears to be richer in phosphoric acid than the yeast flavo-protein of Warburg. Corran and Green believe it activates coenzyme I to pass on its hydrogen just as dehydrogenase activates substrate.

Haas (1938) and Warburg (1938) have also described active flavo-proteins from yeast which are different from the

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original cytoflav, and special interest attaches to a new flavo-protein isolated from animal tissues and called "diaphorase". Straub (1939) has shown that this is a new flavo-protein of high activity, and that it is actually present in muscle tissue.

The study of these flavo-proteins and their action indicates that they are the missing links in the chain of transference of hydrogen from substrates to cytochromes, and that they catalyze the oxidation of reduced coenzymes.

But what are coenzymes and cytochromes?

Coenzymes

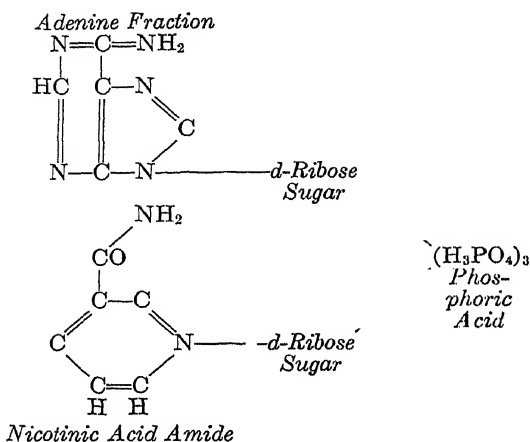
These are hydrogen carriers, but are different from cytochromes or flavo-proteins.

Some time ago an enzyme with ability to ferment sugar was extracted from yeast. It was called "zymase". When the yeast juice containing zymase was filtered through gelatin, both the colloidal part left on the filter and the filtrate were inactive. But when the colloidal matter was combined with the filtrate, reaction took place. This led to the postulation that the colloidal part represented the enzyme proper, and that there was a substance in the filtrate necessary, in addition to the enzyme, to produce the fermentation effect. This filtrate factor was called cozymase or coenzyme I; it contains no protein.

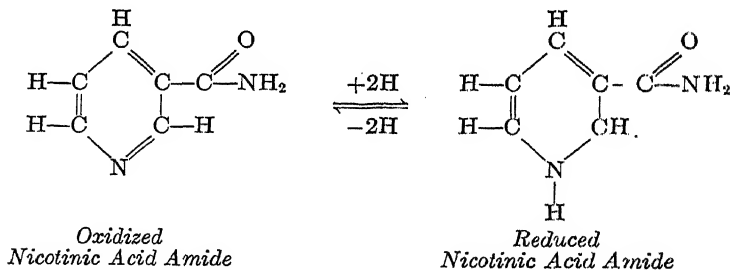
Today two coenzymes, known as I and II, have been isolated, chemically identified and shown to act as hydrogen carriers. The structure of coenzyme II is shown in the figure on the next page.

Coenzyme I, or cozymase, is believed to be similar in structure but to contain only two phosphoric acid groups. Again,

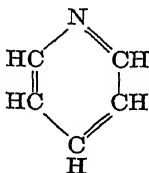
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these coenzymes bring us to vitamins, for one member is nicotinic acid amide, the compound we now know to be the antipellagric vitamin, and it is this member, due to the presence of the pyridine ring, that acts as the hydrogen carrier, *e.g.*,

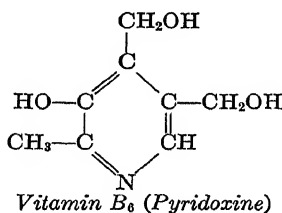


Nicotinic acid and amide contain the pyridine ring



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And the identification of pyridoxine, or vitamin B₆, shows that it also is built on the pyridine ring and hence is also a potential coenzyme former.



Cytochromes

The cytochromes are metal-protein oxidizing substances. Their character is shown in Theorell's structure on page 18. The ability of the metal in such compounds to accept or pass on electrons explains their action in an oxidation system. (See p. 18.)

Phosphorylation

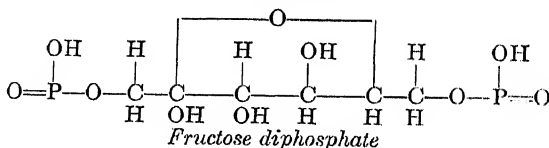
The structure of the coenzyme points to another process of activation necessary to prepare sugars for oxidation, namely a role played by phosphorus. Szent-Gyorgyi showed that the riboflavin part of Warburg's yellow enzyme alone was not effective in hydrogen transport, but had to be combined with phosphoric acid. He called the riboflavin-phosphoric acid part of the enzyme "cytoflav".

It has been known for some time that the first step in the breakdown of sugar in the cell involves its conversion to a hexose phosphate, and that the phosphoric acid for this

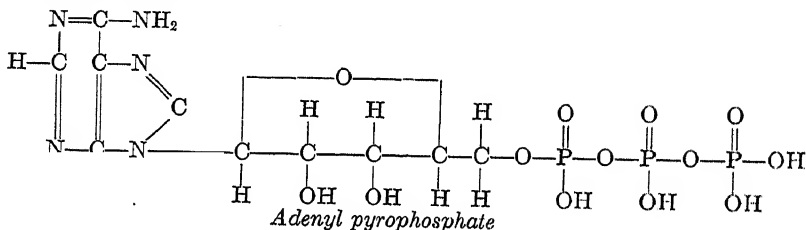
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purpose is derived from adenylyl pyrophosphate, which is changed in the process to adenylic acid and free phosphoric acid.

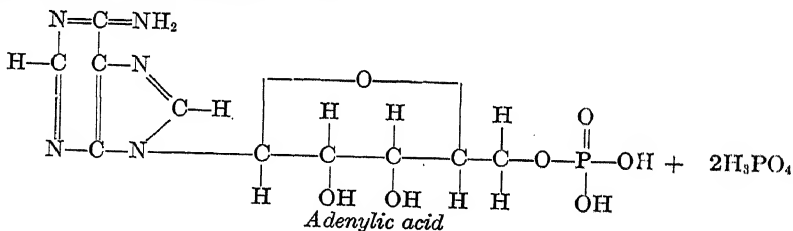
(a) Glycogen + $\text{H}_3\text{PO}_4 \longrightarrow$ Hexose-diphosphate



(b) Phosphoric acid supplied by:



which breaks down to adenylic acid:

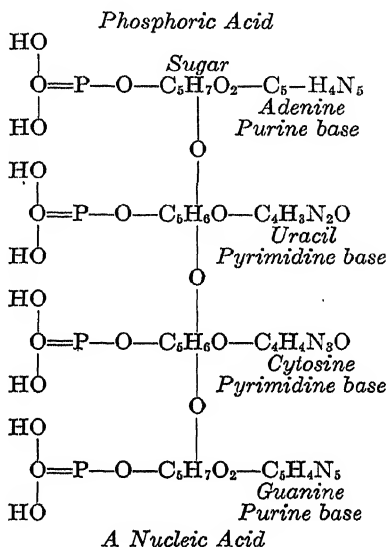


The enzyme phosphatase can break down organic phosphates to liberate free phosphate, and vitamin D is known to control the location of these phosphatases in the body. Again a vitamin (D) appears in direct relation to cellular metabolism.

Still another interesting feature of these coenzymes is that their structure is that of a nucleotide. It has been known for

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some time that the nucleo-protein which characterizes the nucleus of the living cell contains a combination of purine and pyrimidine nucleotides:



The coenzymes are likewise purine (adenine), sugar (ribose), and phosphoric acid nucleotides with a hydrogen carrier, nicotinic acid, attached. It is evident, therefore, that compounds such as nicotinic acid and nicotinic amide and pyridoxine, or B₆, may be the constituents necessary for the formation of these same coenzymes. If that is the case, then, just as lack of vitamin A results in failure to form rhodopsin in the retina of the eye with a resulting loss of visual clearness, so failure of nicotinic acid, pyridoxine and such hydrogen acceptors may, through reduction of coenzyme construction, bring about dysfunction in the tissue cells where these coenzymes operate.

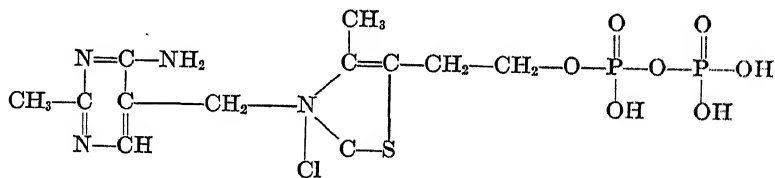
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Carboxylases

When sugar reaches the final stages of carbon dioxide and water production, it becomes evident that the carbon dioxide is formed by splitting it out from acidic compounds, and not by direct union of oxygen with the carbon in the compounds. For example, yeast contains a carboxylase that splits keto-acids such as pyruvic acid into aldehydes, with liberation of carbon dioxide.



The action of this carboxylase, like that of zymase, requires the presence of a co-enzyme or co-carboxylase. Löhman and Schuster (1937) succeeded in isolating such a co-carboxylase from yeast. Analysis of this co-carboxylase showed it to be the pyrophosphate of vitamin B₁ or thiamine pyrophosphate.



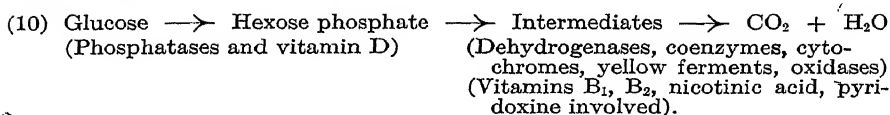
Thiamine pyrophosphate

This discovery again links a vitamin with the processes of cell metabolism. It explains why vitamin B₁ is essential to the breakdown of carbohydrates in the cell and why increased carbohydrate in the diet increases vitamin B₁ need; for the final step in sugar conversion in the cells is transformation of pyruvic acid to carbon dioxide and water.

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Summary

In summary, then, we may picture the transformation of glucose in the cell as involving the following elements (Eq. 10):



Dysfunction due to vitamin deficiency then may be, fundamentally, interference with cellular respiration and metabolism in specific tissues.

Bibliography

- Corran, H. S., and Green, D. E., *Biochem. J.*, 32, 2231 (1938). Flavoprotein from Milk.
- Haas, E., *Biochem. Z.*, 298, 378 (1938); *J. Biol. Chem.*, 130, 425 (1939). Other Flavin Enzymes.
- Löhman, K., and Schuster, P., *Naturwissenschaften*, 25, 26 (1937). Concerning Co-carboxylase.
- Straub, F. B., *Biochem. J.*, 33, 787 (1939). New Flavoprotein.
- Szent Gyorgyi, A., Flexner Lectures, 6th Series, Williams and Wilkins Co., 1939.
- Theorell, H., *Biochem. Z.*, 290, 293 (1937). Structure of Yellow Enzyme. *Biochem. Z.*, 298, 242 (1938). Structure of Cytochrome C.
- Warburg, O., *Science*, 61, 575 (1925). Iron, the Oxygen Carrier of Respiration Enzyme.
- Warburg, O., and Christian, W., *Biochem. Z.*, 254, 438 (1932). Concerning a New Oxidation Ferment and Its Absorption Spectrum. *Biochem. Z.*, 298, 368 (1938). Concerning Other Yellow Enzymes.
- Wieland, H., *Ber.*, 45, 484 (1912). Über Hydrierung und Dehydrierung.

CHAPTER THREE

THE PROPERTIES OF VITAMINS A

THIS vitamin exists in nature in three forms. In plants it is in the form of a yellow pigment called "carotene", which is converted in the animal body into a nearly colorless compound called active vitamin A. Of these active vitamin A forms, two kinds have been identified to date; one, found in the livers of salt-water fish, is known as A_1 , and the other, in the livers of fresh-water fish, is known today as A_2 . An enzyme has been isolated from the liver which is capable of converting the provitamin A, carotene, into the active A_1 or A_2 . It is not yet known whether this conversion can take place in other tissues of the body, but there is some evidence indicating that this is the case.

In foods of vegetable origin the vitamin A is in the provitamin or carotenoid state (alpha, beta, gamma carotene or cryptoxanthin). In foods of animal origin such as butter, milk, cheese, fish oils, etc. it may be present in either the provitamin form or in the converted, active A form; or both forms may be present. (For distribution in common foods see Appendix B.)

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General Properties

The Standard U. S. Pharmacopeia test for vitamin A potency is based upon the ability of this vitamin to restore growth to test animals (rats) depleted of this vitamin. This, however, is what we may call a non-specific function of vitamin A, since deprivation of any vitamin or any nutritive factor essential to the animal's nutrition will, of course, arrest growth and cause weight loss.

In the advertising of vitamin A sources such as fish-liver oil and concentrates, stress has been put on its relation to cold prevention, not because vitamin A will act definitely on infective organisms, but because colds are a form of infection familiar to everyone, and hence the claim is made because of the ability of vitamin A to produce resistance to infections in general. E. Mellanby (1934) suggested that because of this relation to infection vitamin A be called the "anti-infective vitamin." There has been general objection to this term for the specific reason that vitamin A does not act as an immunizing body. Rather, it accomplishes its anti-infective effect only by maintaining a healthy and hence more germ-resistant tissue.

One of the earliest manifestations of vitamin A deficiency to be definitely related to inadequate supply of the vitamin was an eye disease called xerophthalmia or "dry eye". This disease developed in Japan and in Denmark as a result of a reduction of the milk supply to infants, and has caused this vitamin to be sometimes called the anti-xerophthalmic factor.

A later discovery was that of the relation of vitamin A to the visual purple and visual violet in the rods and cones of the eye. It has been shown that these pigments are satisfac-

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torily regenerated only when vitamin A is available in adequate quantities as a building material. Loss of visual acuity follows such deprivation of vitamin A and is measurable by suitable instruments. Such measurements are now being used to help determine the adequacy of the individual's vitamin A intake.

Frazier and Hu (1936) were the first to point out changes resulting in a particular form of skin dryness that resulted from A deficiency, and topical applications of vitamin A or vitamin A therapy have recently challenged attention as a means of correction of this condition.

What do we know of the actual behavior of vitamin A to substantiate its relation to these deficiency defects?

Metaplasia

Epithelial tissues are layers of cells covering various parts of the body, both internal and external. Change in the structure of these tissues from one form to another is known as metaplasia, and is producible by deficiency of vitamin A.

The specific anatomic effect of inadequacy of vitamin A in the rat, guinea pig, monkey, chicken and other animals is the loss of ability to maintain certain epithelial surfaces. The result is the replacement of such surfaces by specialized cells. These changes may be localized in various regions of an epithelial layer, or may spread over a considerable part of the area. In the same animal there is usually a progressive spread of the effect (Wolbach and Howe, 1925), the order being first the respiratory mucous covering, the salivary glands, the eyes, the lining of the gastro-intestinal tract,

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the parocular glands, the pancreas and the liver. Eye lesions were not found in the monkey or the guinea pig.

In the early stages of the deficiency, areas of darkly stained epithelium are seen to undergo rapid growth. As they grow, the overlying epithelium degenerates and sloughs off, and islands of stratified epithelium are formed. The phenomenon implies that in vitamin A starvation some material absolutely essential to the epithelial cells' normal form is lacking.

The primary consequence of this change is a loss of function of the affected surface. In the case of the trachea, the loss of cilia prevents proper cleansing of that part, and in the conjunctiva there is a loss of mucus-secreting cells with a similar effect. Another effect is the blockage of gland ducts, which leads to stagnation of secretory flow and ultimately to infection. The question of whether infection occurs or not depends in part on the accessibility of infected parts to bacteria; and for that reason association between abscesses and metaplastic changes from vitamin A deficiency have been most commonly found about the mouth and its glands. Their exposure to infection is obviously easy.

Wolbach (1925) describes the changes in the epithelial cells as follows: First, they start to atrophy; then comes proliferation of basal cells and differentiation into a stratified, keratinized or horny form. As these cells grow they undermine the atrophied cells and ultimately cause them to slough off. The hornified cells replace them.

When this condition has developed and vitamin A treatment is given, repair proceeds in the reverse order. First there is a separation of the hornified layer and vacuoling of the cells of the intermediate layer. The upper zone degenerates, the hornified cells die and are pushed off, and their

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place is taken by the deep zone cells which are now normal and non-keratinized.

There is also evidence to indicate that whatever essential it is that vitamin A contributes to the epithelial cells, it also has an effect on the rate of their proliferation. Rowe and Dalldorf (1937) were able to determine by the cultivation of chick iris epithelial cells *in vitro* that the rate of proliferation could be reduced by removal of vitamin A from the nutrient plasma, and also that it could be accelerated by increasing that concentration within certain limits. These tests confirm the view expressed by Lohr (1937) and others that the healing effect of cod liver oil on burns and wounds may be due in part, at least, to its vitamin A content.

Relation to Eye Function

Our eyes make objects visible to us by focussing the light rays on the retina of the eye-ball. This focussing is accomplished by the eye lens, just as the camera lens focusses images on the photographic plate or film. The retina of the eye corresponds to a camera plate or film.

In order to get the picture formed by this action of the light rays on the camera film, we have to develop it. The picture formed by the light rays on the retina is "developed" by the production of nerve impulses which travel from the retina over the optic nerve to the brain and are there translated into vision. In the photographic film the image is formed by chemical changes in the photographic film, usually the change of a silver salt to silver. The production of ocular nerve impulses in the retina of the eye is accomplished by

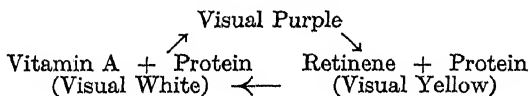
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chemical changes of similar nature, and the pigments involved are distributed in the rods and cones of the retina.

In the rods is a pigment called rhodopsin, or *visual purple*, and in the cones a pigment called *visual violet*. The impingement of light rays on visual purple bleaches it to a yellow substance called retinene. It is this bleaching of the visual purple which produces the nerve stimulus. Once bleached, this pigment cannot again respond to light until it has been changed back to visual purple. It is in this regeneration of retinene to visual purple that vitamin A functions.

Various observers (Fredericia, 1925; Yudkin, 1931; Akroyd, 1930; Wald, 1935-36) had reported finding vitamin A or carotene present in the retinas of different kinds of animals. Later study showed that vitamin A and carotene were used in the regeneration of visual purple, and still later (Hecht, 1939), showed that the visual violet of the cones is also dependent on adequate supply of vitamin A for its regeneration.

The steps in the use of vitamin A for the regeneration of retinene are pictured in the following diagram:



It is obvious, therefore, that the rate of regeneration of visual purple may be an index of the rate of supply of vitamin A to the eye. If failure of such supply is due to lack of vitamin A, measurement of visual acuity may then become an index of adequacy of vitamin A in the diet. Instruments have been devised to measure degrees of visual acuity, and conclusions drawn from such tests as to vitamin A needs or vitamin A adequacy of the diet (Jeans, 1937; Jeghers, 1937).

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It must be borne in mind, however, that lack of adequate supply of vitamin A to the eye might be due to other causes than lack of vitamin A in the food intake. If for any reason there is failure of absorption from the digestive tract, such as might occur in conditions of colitis for example, there will occur a lack of supply to the eye in spite of large intake. Unless the feeding of vitamin A after revelation of lowered visual acuity promptly restores the condition to normal, such tests should not be used as certain indication of diet inadequacy or individual requirement (Isaacs *et al.* 1938).

Consequences of Metaplasia

As previously noted, metaplasia due to vitamin A deficiency occurs in epithelial tissues. What are the consequences of changes in the body regions lined or covered with epithelial cell layers?

Gastro-intestinal Tract

Bessey and Wolbach (1938) state that metaplasia of the lining of the gastro-intestinal tract due to vitamin A deficiency is comparatively rare in man:

“Lesions of the stomach and intestine in association with vitamin A deficiency are of rare occurrence in man and in experimental animals and beyond slight degrees of atrophy of mucosa, are probably not related specifically to the deficiency.”

Such changes have, however, been reported. Fehr (1920) reported keratomalacia, with atrophy of the intestinal tract; Cramer (1921) reported atrophy of intestinal villi in experimental animals on A-deficient diet.

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Manville (1933, 1938) claims that secretion of gastric mucus is decreased by A deficiency but not the flow of gastric juice. In the absence of this mucus and with the acidity of the gastric juice, such an effect might be responsible for the production of peptic ulcers through failure of protection of the lining from irritation. The evidence in general, however, suggests that whatever disturbances are produced in gastro-intestinal action by vitamin A deficiency they are of functional rather than anatomical origin.

Conditions in the gastro-intestinal tract may, however, seriously interfere with the absorption of vitamin A from the tract into the liver and the circulating blood.

The two active forms of vitamin A are alcohols. In the presence of fatty acids they may be converted into esters. Such esters require the presence of bile to secure their absorption; hence a diminished flow of bile, or other conditions unfavorable to fat absorption, hinders the absorption of vitamin A itself. (Altschule, 1935; Greaves, 1935).

The hydrocarbon carotene cannot form esters, which may account for its less ready absorption (Clausen, 1938). The relative absorbability of carotene, vitamin A esters, and the vitamin A alcohol forms is not fully established and needs further study.

As noted above, interference with the flow of bile and a deficiency of bile salts may produce failure to absorb vitamin A. Obviously diarrhea, pancreatic dysfunction or any of the factors which interfere with fat digestion may therefore also interfere with vitamin A absorption.

Mackie and Eddy (1939) have shown that in certain cases of ulcerative colitis there was marked reduction of vitamin A absorption in spite of high dosage of the vitamin

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as measured by blood content of that factor, and that in such cases it was possible to get almost immediate rise in the blood vitamin A through topical application of cod liver oil to the chest region of the patient.

The failure of vitamin A absorption from the digestive tract is probably not explicable by metaplasia of the lining membrane cells but to other conditions in that tract unfavorable to A absorption. Such conditions may make any amount fed unavailable to other parts of the body. This is another reason why vitamin A deficiency, as revealed by visual acuity tests, should be checked by other tests before drawing the conclusion that the diet was inadequate in that factor.

We have noted above that active vitamin A has been reported to be more effectively absorbed than the provitamin carotene, but both are effectively absorbed under normal conditions. The absorbed carotene is converted, in part at least, in the liver to active vitamin A. It may also circulate in the blood without change to active A. This transformation in the liver is believed to be due to an enzyme (carotenase) demonstrated to be present in liver. Whether this enzyme is present in other than liver tissue is as yet undetermined.

It is also possible that the thyroid gland plays a role in converting carotene to A. Cattle and goats secrete vitamin A into their milk. White milks run high in vitamin A and low in carotene. Yellow milks contain carotene as well as active A, and in such milks it is assumed that the cow's ability to change carotene is less efficient than in cows producing white milks. Goats usually produce white milks containing A, but little or no carotene. Goats with thyroids removed changed this condition and produced yellow milk.

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This suggests a possible thyroid gland involvement in carotene conversion.

Conversely, it is also known that in the presence of mineral oil, vitamin A is less readily utilized than carotene, presumably because the latter is less soluble in mineral oil.

We still have much to learn of the behavior of forms of vitamin A in gut and in liver and the factors that control its behavior. Mackie and Eddy (1940) found that, in cases of peptic ulcer showing a low blood vitamin A and C, increased feedings of these vitamins produced an immediate increase in blood C but not a similar increase in blood A. Diabetic cases (Ralli, 1936) show high blood carotene and reduced ability to convert the carotene to vitamin A.

The evidence, then, indicates that the anomalies in the absorption and conversion of carotene to vitamin A cannot be explained by metaplastic changes in the epithelial lining of the gastro-intestinal tract and that we still lack explanation of why fed carotene and A, in certain conditions, fail to reach the circulating blood supply and the tissues where they are needed.

Metaplasia of the Respiratory Tract Linings

It is generally conceded today that colds probably start with infection by a specific virus. This infection is accompanied by congestion of nasal and respiratory passages and under these conditions secondary invading germs may develop and thrive; especially if the surface of the respiratory passage is so changed as to permit the lodgement and penetration of these secondary invaders. Such roughening and changes of epithelial surfaces do take place in the respiratory mucosa

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in vitamin A deficiency, their development under conditions of A deficiency being increased by the stimulation of irritants of various sorts (McCullough and Dalldorf, 1937).

We may, then, today consider that when infection in the respiratory tract follows vitamin A deficiency, one cause of such infection may be metaplastic change in the epithelial linings of those passages, which make them more permeable to the germs which lodge on their surfaces. Metaplasia of the epithelium lining of the ducts of glands opening into the respiratory tract, such as the salivary glands, may clog these ducts and permit the incubation of germs within the glands or regions surrounded by the metaplastic epithelium.

It is not surprising, then, that there has been variation in the effects of vitamin A in attempted prevention of colds in the hands of different experimenters (Shibley and Spies, 1934, Beard, 1934, Cameron, 1935). Most investigators are now agreed that vitamin A dosage shows little value in preventing the incidence of a cold, but that those so infected tend to recover more quickly if they have built up reserves of this vitamin.

The Council of Pharmacy of the American Medical Association states its viewpoint for vitamin A claims of potency as follows:

"Present indications are that vitamin A is an aid toward establishing resistance of the body to infections in general only when there has been a decrease in body reserves of the vitamin and the ingestion of vitamin A is inadequate. It has not been shown to be specific in the prevention of colds, influenza, and such infections, nor has it been demonstrated that ingestion of vitamin A far in excess of that necessary for normal body function, and readily obtained from a properly selected diet, is an aid in preventing various types of infections."

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Since, however, all are agreed that the cells of the lining of the respiratory tract do undergo metaplasia in vitamin A deficiency and that such areas of modified cells become thereby ports of entry for germs, it is not unreasonable to urge attention to vitamin A adequacy to keep the respiratory tract lining at its most efficient condition for resistance to germ invasions. It is equally certain today that vitamin A is not an immunizing substance and has no ability to destroy or inactivate any type of germ.

Metaplasia in Mouth and Ear

In experimental animals such as the rat, one of the earliest manifestations of vitamin A is pus formation in the salivary glands at the base of the tongue. Donnell (1938) has reported successful treatment of infection of the middle ear (otitis media) by local use of wicks and liberal supplements of cod or halibut liver oil. That such infections follow metaplasia of the epithelial linings of these regions due to A deficiency and subsequent occlusion of ducts and infection is now well established.

Metaplasia of the Tooth Enamel Organ

Tooth enamel is an epithelial structure; its formation is controlled by what is called the enamel organ. In vitamin A deficiency, metaplasia in this organ results in the enameloblasts being replaced by stratified, keratinized epithelium. This is followed by a loss in enamel and exposure of the dentin, which gives the teeth a chalky appearance. Simultane-

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ously, according to Wolbach and Howe (1925, 33, 37), the odontoblasts which form the dentin of the tooth atrophy and tooth growth ceases. Vitamin A, then, is essential to the proper formation and maintenance of tooth enamel.

Metaplasia of the Skin

The entire outside of the body is covered with an epithelial layer, and in consequence one would expect vitamin A deficiency to manifest itself in metaplasia of this tissue. Such metaplasia in children has been recognized for some years.

Youmans and Corlette (1938), reviewing twenty cases, report that the skin is actually one of the first tissues to show vitamin A deficiency and that at least four weeks of oral vitamin A therapy was necessary to restore the skin to normal conditions. One of the characteristic manifestations of A deficiency in skin is hyperkeratosis (excessive hornifying) of the epithelial lining of the hair follicles, which results in their becoming plugged with masses of hornified epithelial material. In consequence, the flow of the secretions of the oil glands over the surface of the skin is interfered with. The outstanding result of this is dryness of the skin, which obviously cannot be corrected by mere lubrication of the skin tissue.

The first to report skin lesions were Nicholls in India (1934) and Frazier and Hu (1931) in China. They report that the keratotic hair follicles may manifest themselves on the surface by papules which are dark gray in color and often surrounded by grayish pigmentation. In acute cases the skin may show projections giving it a "toad skin" appearance which has been characterized as phrynoderma.

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Lowenthal (1933, 1935) has stated:

“Subsequent monthly inspections of persons with new cases of dermatoses were recorded and it was found that the majority of these men suffered from night blindness and xerophthalmia while almost every sufferer from xerophthalmia and night blindness showed these cutaneous changes.”

The skin lesions seen by Lowenthal “presented the clinical picture of acne vulgaris with a dermatosis which none of the medical officers present could define.” It would appear that the effect of vitamin A deficiency on skin is fairly common and is an early manifestation of this deficiency; that the reason for lack of record of such changes is due to failure to observe them rather than to their absence.

The slow response of skin metaplasia due to A deficiency to oral administration of vitamin A has focused attention on the possibility of topical application of the vitamin as a quicker acting procedure. It has been demonstrated by Eddy and Howell (1938) that vitamin A and carotene can both be absorbed *through* the skin to produce a systemic effect; also that when the same quantities are given by mouth and by topical application, systemic effect is less with the topically applied material. Does this mean that in the topical treatment some of the A, or carotene, remains in the skin epithelium and can thus function therein immediately, without having to travel to the liver and out again over the circulatory system?

We know from the *in vitro* culture experiments of Dall-dorf and Rowe with iris epithelium that epithelial cells bathed in a solution of vitamin A or carotene can absorb

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and utilize it, and we know that the active epithelial cells of the epidermis are in contact with lymph-filled spaces.

We lack conclusive evidence as to whether vitamin A or carotene reaching these spaces is actually absorbed from them into the cells without first going to the liver; but a recent aid to the study appears to be available. Popper and Greengard (1940) have shown that when epithelial cells contain vitamin A, the droplets of A can be made to fluoresce by exposure to ultraviolet rays and thus become visible under the microscope. With their instrument it should be possible to examine sections of skin both topically treated and treated by oral ingestion of vitamin A and determine the relative cell content. Until such tests have been made we cannot conclusively determine which is the more effective method of treating skin keratosis.

The question of the value of topical application of vitamins A and D has received special attention in connection with the treatment of wounds by cod liver oil and by lotions containing vitamin A and D. Löhr (1937), in particular, has studied this problem extensively, and it has also been reviewed and studied by a considerable number of other investigators. There seems to be no question that vitamin A-containing oils, like cod liver oil, and lotions have proved beneficial in increasing the rate of wound healing. The investigators in this field incline to the view that the principal agents of the dressings in producing this healing effect are vitamins A and D. It is perhaps too early to reach absolute conclusions on this point, but the problem is under active study at the present time. Eller and Wolff (1940) have reviewed the evidence in part.

Referring again to the studies of Dalldorf and Rowe

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(1937), these investigators, by growing chick iris epithelial cells *in vitro* and using chick embryo juice as a culture medium, demonstrated that the removal of vitamin A from the culture medium produced a cessation in the growth of the epithelial cells, and—that reinforcement of the medium with additional amounts of vitamin A up to certain limits resulted in increased rate of epithelial cell proliferation. Their work demonstrated clearly that vitamin A could pass directly from the culture medium into the tissue cells suspended in that medium. A corollary to this would be that contact of a vitamin A lotion with epithelial cells should be sufficient to allow the passage of vitamin A from the lotion into the tissue cells without the intervention of circulating blood. The present availability of creams, unguents, and lotions containing vitamin A should make it possible to determine this question of the desirability of topical application and its efficiency relative to oral dosage.

Metaplasia in the Eye Regions

Bloch (1921, 1931), and Wright and Mori (1922, 1923) first described the condition now known as xerophthalmia and established its relation to vitamin A deficiency. We know today that this xerophthalmia is a late, rather than an early, manifestation of vitamin A deficiency and that it is preceded by metaplasia of the epithelium of the conjunctiva and cornea. Tear glands also atrophy and tears cease to wash the eyeball. The cornea becomes bloodshot and swollen; ulceration and even perforation of the eyeball may ensue. The recognition of vitamin A deficiency in this

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condition is responsible for the early use of the term "anti-xerophthalmic vitamin" for vitamin A.

These ocular lesions usually commence as small, dry, round, or triangular patches at the angles formed at the junction of the eye lids. They are known to the diagnostician as Bitôt's spots. They are formed of cornified epithelia and collected bacteria. Hardening of the cornea is followed by changes in the middle layers accompanied by infection, liquefaction and ultimate destruction of the cornea. Similar changes occur in the conjunctival epithelia, and there may also be development of a peculiar pigmentation in the conjunctiva. If the case is not too far developed, these cases respond promptly to vitamin A treatment. It was this fact, and the failure to respond to the treatment with disinfectants, that proved the direct relation to vitamin A as a causal factor.

Pillat (1929) has described the progressive effects of continued vitamin A deficiency on the eye as follows:

1. Hemeralopia (inability to see clearly in dim light) earliest symptom.
2. Appearance of Bitôt's spots.
3. Further hardening of areas of the cornea and conjunctiva.
4. Softening of the cornea or keratomalacia.
5. Lid irritation, causing winking.
6. A decrease in flow of tear fluid.
7. Swelling and puffiness of the eye-lids.

These xerophthalmic changes occur much later than changes in visual acuity (hemeralopia or night blindness). Hemeralopia is due to inability to regenerate visual pigments; xerophthalmia, to metaplasia.

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Metaplasia of the Genito-Urinary Tract Lining

The mucus membrane lining of the uterus is an epithelial structure and is called the endometrium. Wilson and DuBois' (1923) autopsy on a vitamin A-deficient child body demonstrated keratinization in the renal pelvis. Bloch (1931) and Spence (1931) noted pus formation in that region in cases of A deficiency and McCullough and Dalldorf (1937) found that sex hormones induced keratinization in rats on A-deficient diet but not in rats on A-adequate diets.

That A deficiency produces metaplasia of the endometrium and definite changes in the genito-urinary tract is fully established and has been ably reviewed by Mason and Wolfe (1935). Briefly, they found atrophy of the testes, placental injury which results in prolonged gestation, difficult parturition and excessive uterine bleeding. This effect is quite different from that resulting from vitamin E deficiency.

In E deficiency it is the fetus which suffers from vitamin lack. In A deficiency it is the *nutrition* of the fetus that is impaired and its growth retarded by changes in the placenta. Female rats deprived of vitamin A show abnormal estrus and the persistence of cornified cells in the vaginal epithelium. It is evident, then, that a generous supply of vitamin A during pregnancy is indicated by these findings to avoid placental injury and interference with the nourishment of the developing infant.

Moore (1936) has reported production of lesions in the prostate gland of rats on a vitamin A-deficient diet. In the genitals, as elsewhere in the body, it is the metaplasia of the epithelial structures which is preliminary to the development of infection or change of function in the regions affected.

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There is active controversy at present as to whether bladder or kidney stone (urinary lithiasis) is a direct effect of vitamin A inadequacy. Mendel (1932) was one of the first to note that bladder stone was fairly prevalent in the rats maintained on vitamin A-free diets.

The American Medical Association's Council of Pharmacy reviewed the evidence of relation of vitamin A to lithiasis up to 1938 and announced the following conclusion:

"The existing evidence does not warrant claims for the use of any of the vitamins and particularly vitamin A in prevention or treatment of urinary lithiasis."

The problem of the cause or causes of urinary lithiasis are, however, still undemonstrated. That A deficiency might be one of such causes seems indicated by the work of the investigators in this field. Present attitude on this point therefore would seem to be recognition of need for further study rather than positive denial of possible connection with A deficiency.

Vitamin A and the Nervous System

Mellanby (1935) insists that vitamin A deficiency produces specific lesions in the nerve tissues and even suggests that some of the changes in the epithelial tissues are the consequence of such nerve derangements. Nerve lesions have been demonstrated in vitamin A-deficient subjects (Zimmerman, 1933; Sutton, 1934). Suzman (1932) was, however, unable to confirm Mellanby's earlier findings, and Bessey and Wolbach (1938) definitely contradict his claims of the effect of A deficiency on nerve tissue. The feeling of the objectors to his theory is that while nerve lesions may occur

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they are the result of malnutrition rather than a specific effect of vitamin A deficiency on the nerve tissue. This problem, like many others in the vitamin field, is a matter of controversy and there is lack of conclusive evidence to determine the correct viewpoint.

Vitamin A and the Blood System

We have no satisfactory evidence of any relation between vitamin A and the prevention of nutritional anemia. However, nutritional anemia is a frequent accompaniment of malnourishment and consequently a generous supply of vitamin A to malnourished individuals would seem to be indicated as desirable for getting them back into a well-nourished state, the indirect effect of which would be correction of some of the derangements associated with malnutrition.

Diagnosis of Vitamin A Deficiency

It is generally agreed that one of the earliest signs of A deficiency is hemeralopia or night blindness. The development of instruments to measure visual acuity as a diagnostic means of determining A deficiency has therefore been rapid.

Jeans and Zentmire (1936, 1937) used biophotometer readings (hemeralopia measuring instrument) to determine the minimum vitamin A requirement for eleven-year old boys, and as a result put the figure at 3000 I.U. per day. Jeghers (1937), working with adults and the biophotometer, fixed the minimum requirement for prevention of hemeralopia at 4000 I.U. per day and recommended 6000.

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But criticism of such conclusions began to develop. Isaacs *et al.* (1938) reported poor correlation between visual acuity readings and vitamin A intake. Such criticisms have had two effects. First, they have resulted in improvement in the methods of making the test. Secondly, they have forced recognition that a low visual acuity test may be due to other causes than faulty vitamin A intake.

In connection with development of methods of test, Hecht (1939) has postulated six specifications which he says must be met if the measurements are to be numerically precise and quantitatively valid:

1. Control of intensity of the light.
2. Control of the duration of the light present before dark adaptation begins.
3. Control of the area studied.
4. Control of the retinal location.
5. Control of the color of the light.
6. Control of the duration of the light used for measuring the course of dark adaptation.

In response to the second point raised, the hemeralopia test is today considered truly indicative of low vitamin A intake only when feeding A can be shown to produce cure. To determine where the failure to maintain normal vision lies when feeding A fails to correct it requires other diagnostic measures. Study of blood content has been one means of handling this problem.

Carr and Price (1926) worked out a colorimetric method of estimating vitamin A by causing it to produce a blue color with antimony trichloride, and by comparing the

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intensity of the blue color produced by a given amount of vitamin A source against standards prepared by similar treatment of known amounts of vitamin A. In the early experiments these color standards were matched against certain color plates in the Lovibond Tintometer, permitting the use of that instrument for comparisons without necessity of preparing fresh standards, and it became customary to express vitamin A potency in terms of Lovibond blue units.

The blue color produced by antimony trichloride is transitory, in other words, fades rapidly. This difficulty has been met by the use of the photoelectric cells to catch the color at peak intensity; photoelectric colorimeters are available for this purpose at the present time.

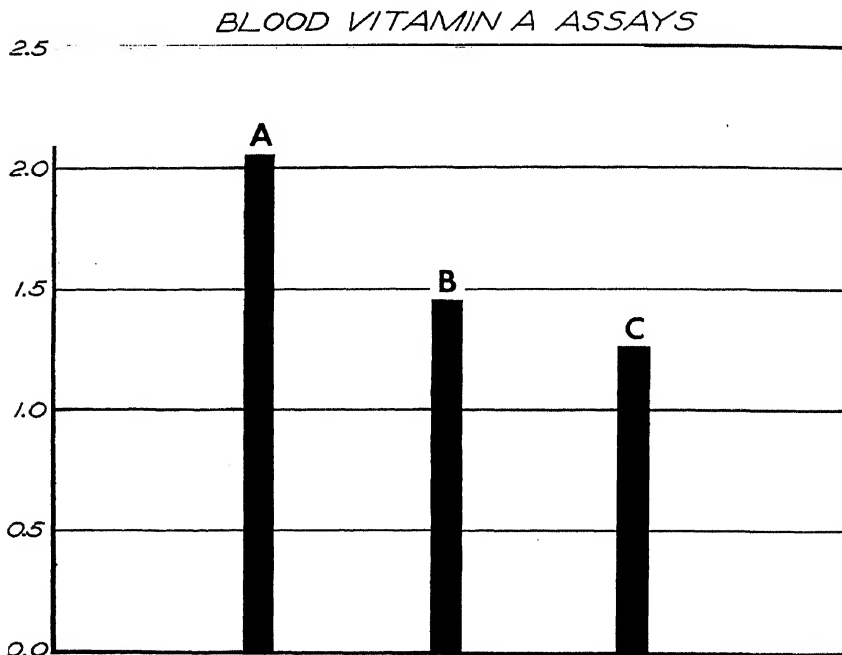
Carotene produces a color reaction with antimony trichloride which can be differentiated from that of pure vitamin A or vitamin A ester (Ferguson, 1935; Clausen and McCoord, 1936); but carotene is also itself a yellow pigment. It has been found possible to extract carotene quantitatively from the source and match intensity of the yellow coloration obtained against tubes containing definite amounts of pure carotene or dichromate solution. When such tubes are matched against the yellow plates of the Lovibond Tintometer, that instrument can again be used for measuring carotene content with or without a photoelectric cell to increase sensitivity. Consequently carotene units are frequently expressed as Lovibond yellow units.

It was found possible to adapt these colorimetric measurements to examination of blood and other biological fluids and considerable amounts of data have already been collected to make possible comparisons between apparently

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normal individuals and those under specific disease conditions.

Menken (1932), Clausen (1933), Ralli and Heyman (1936), Stepp and Wendt (1937) and Steininger, Roberts



A = Average of 39 Fasting Normals

B = Average of 66 Peptic Ulcer Cases

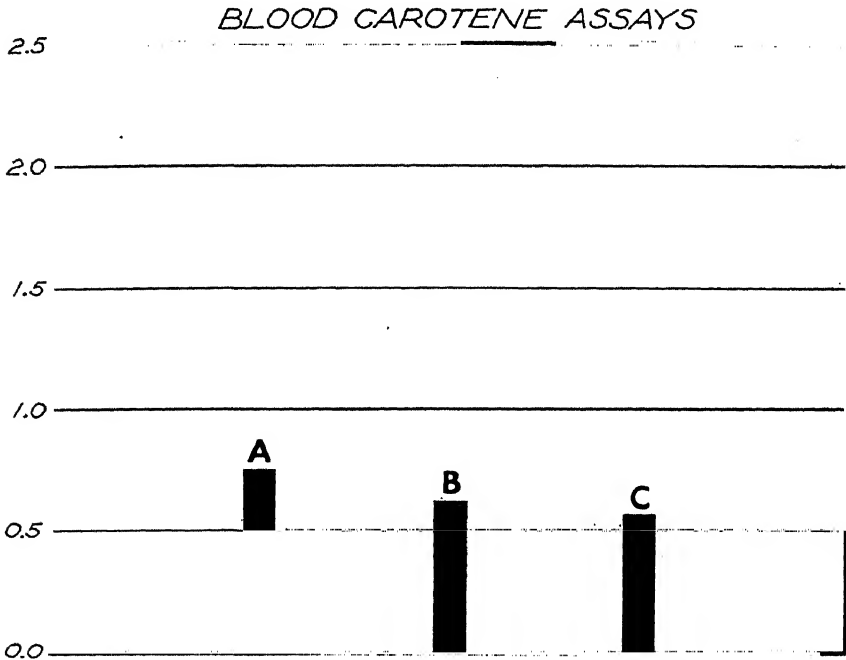
C = Average of 40 Ulcerative Colitis Cases

(Mackie and Eddy, 1939)

and Brennan (1939) have all reported findings on either carotene or vitamin A by such methods. The main difficulty in comparing these results is the difference in the method of

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establishing unitage; however, they throw some light on the blood distribution of these factors. Regardless of unitage used or method, and regardless of whether these methods



A = Average of 38 Fasting Normals

B = Average of 67 Peptic Ulcer Cases

C = Average of 40 Ulcerative Colitis Cases

(Mackie and Eddy, 1939)

are 100% quantitatively active, the use of a given method for comparison of groups becomes significant.

Mackie and Eddy (1939) conducted such a series of blood

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A and blood carotene tests by the method of Menken (1932) with the following results:

Table 4. Comparative Blood Vitamin A and Carotene Tests.

Data	Vitamin A Assays			Carotene Assays		
	Fasting Normals	Peptic Ulcer Cases	Colitis Cases	Fasting Normals	Peptic Ulcer Cases	Colitis Cases
No. Cases	38	66	40	38	65	40
Mean Finding	2.06	1.45	1.26	0.75	0.62	0.56
Sq. Mean Error	0.28	0.26	0.09	0.085	0.078	0.066
Mean Error Single Obs.	0.53	0.51	0.30	0.292	0.279	0.257
Standard Deviation	0.086	0.062	0.047	0.047	0.034	0.040
Probable Error	0.058	0.042	0.031	0.0307	0.023	0.027

A. Blood A comparisons.

Difference between means. Normals and Ulcer Cases =
 $8 \times$ probable error

Difference between means. Normals and Colitis Cases =
 $12 \times$ probable error

B. Carotene Comparisons

Difference between means. Normals and Ulcer cases =
 $3 \times$ probable error

Difference between means. Normals and Colitis cases =
 $4 \times$ probable error

See Chart for graphic comparisons and individual variations.

These same results are shown graphically in Chart 1.

It will be recalled that in discussing the effect of vitamin A on the gastro-intestinal tract, certain investigators have suggested that though no lesions of stomach and intestine have been satisfactorily demonstrated, there is evidence of vitamin A deficiency in peptic ulcer and colitis cases. The results

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cited above will tend to support this viewpoint since, in the case of peptic ulcer cases at least, these individuals were on a rather high milk diet and the low values could not be attributed to lack of adequate amounts of vitamin A in the diet at the time the tests were made.

To what extent blood determinations indicate normality, and what is the normal blood content of these two factors remain to be established. It has been shown in diabetes, for example, that the carotene is usually abnormally high and the vitamin A/carotene ratio considerably less than in normal individuals (Heyman and Ralli, 1936).

With such blood tests and greater use of them in clinical laboratories, correlated with clinical observations, we should have in time satisfactory clinical methods for estimating vitamin A needs and what constitutes body saturation of this factor, and also a method of measuring directly the absorbability of a given dosage of the vitamin material. The availability of pure vitamin A esters also promises aid to the physician in securing precision in his studies of response to vitamin A therapy.

Human Needs for Vitamin A

It is generally agreed that the average adult needs at least 4000 International units of A per day for maintenance of normality and 6000 are recommended. Amounts over this are not toxic.

Bibliography

- Akroyd, W. R., *Lancet*, **1**, 824 (1930). Vitamin A and Retina.
Altschule, M. D., *Arch. Path.*, **20**, 845 (1935). Vitamin A Deficiency in Congenital Atresia of Bile Ducts and Jaundice.

WHAT ARE THE VITAMINS?

- Beard, H. H., *J. Am. Dietetic Assn.*, 10, 193 (1934). The Prophylactic Effect of Vitamins A and D Upon the Prevention of the Common Cold.
- Bessey, O. A., and Wolbach, S. B., *J. Amer. Med. Assn.*, 110, 2072 (1938). Vitamin A: Physiology and Pathology.
- Bloch, C. E., *J. Hyg.*, 19, 283 (1921). Clinical Investigation of Xerophthalmia.
- Bloch, C. E., *Am. J. Dis. Chil.*, 42, 263 (1931); 27, 139 (1924). Effects of Deficiency in Vitamins in Infancy.
- Cameron, H. C., *J. Am. Dietetic Assn.*, 11, 189 (1935). The Effect of Vitamin A Upon the Incidence and Severity of Colds Among Students.
- Carr, F. H., and Price, E. A., *Biochem. J.*, 20, 497 (1926). Colour Reactions Attributed to Vitamin A.
- Clausen, S. W., *J. Amer. Med. Assn.*, 101, 1384 (1933). Limits of the Anti-infective Value of Provitamin A. *J. Amer. Med. Assn.*, 111, 144 (1938). The Pharmacology and Therapeutics of Vitamin A.
- Clausen, S. W., and McCoord, A. B., *J. Pediatrics*, 13, 635 (1938). The Carotenoids and Vitamin A of the Blood.
- Cramer, W., Drew, H. H., and Mottram, J. C., *Lancet*, 2, 1202 (1921). On the Function of the Lymphocyte and Lymphoid Tissue in Nutrition.
- Donnell, H., *Texas State Med. J.*, 34, 39 (1938). Chronic Otitis Media; Conservative Treatment.
- Eddy, W. H., and Howell, J., *N. Y. State J. Med.*, 39, 406 (1939). Topical Application of Vitamin A.
- Eller, J. J., and Wolff, S., *J. Amer. Med. Assn.*, 114, 1864, 2002 (1940). Hormones and Vitamins in Cosmetics.
- Fehr, E., *Lehrbuch der Kinderheilkunde*, 6th Ed.
- Ferguson, W. S., *Analyst*, 60, 680 (1935). Curves for Use in the Colorimetric Estimation of Carotene.
- Frazier, C. N., and Hu, C. K., *Arch. Int. Med.*, 48, 507 (1931). Cutaneous Lesions Associated with a Deficiency of Vitamin A in Man.
- Fredericia, L. S., and Holm, E., *Am. J. Physiol.*, 73, 63 (1925). Influence of Deficiency of Fat-Soluble A in the Diet on the Visual Purple in the Eyes of Rats.
- Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 111, 482 (1935). On the Absorption and Utilization of Carotene and Vitamin A in Choledochocolonostomized Vitamin A-Deficient Rats.
- Hecht, S., Harvey Society Lectures, 1937-1938. *Physiol. Reviews*, 17, 239 (1937). Theory of Vision.

VITAMINS A

- Hecht, S., and Mandelbaum, J., *J. Amer. Med. Assn.*, 112, 1910 (1939). The Relation Between Vitamin A and Dark Adaptation.
- Heyman, W., *J. Amer. Med. Assn.*, 106, 2050 (1936). Carotenemia in Diabetes.
- Isaacs, B. L., Frederic, T. J., and Ivy, A. C., *J. Am. Med. Assn.*, 111, 777 (1938). Vitamin A Deficiency and Dark Adaptation.
- Jeans, P. C., and Zentmire, Z., *J. Amer. Med. Assn.*, 106, 996 (1936). The Prevalence of Vitamin A Deficiency among Iowa Children.
- Jeans, P. C., Blanchard, E., and Zentmire, Z., *J. Amer. Med. Assn.*, 108, 451 (1937). Dark Adaptation and Vitamin A; a New Photometric Technique.
- Jeans, P. C., Am. P. H. Assn. Address, 1938. Unpublished.
- Jeghers, H., *J. Am. Med. Assn.*, 109, 756 (1937). The Degree and Prevalence of Vitamin A Deficiency in Adults. *Ann. Int. Med.*, 10, 1304 (1937). Night Blindness as a Criterion of Vitamin A Deficiency.
- Lohr, W., and Unger, F., *Arch. f. klin. Chir.*, 189, 405 (1937). Animal Experimentation in Wound Healing.
- Lowenthal, L. J. A., *Arch. Dermat. and Syph.*, 28, 700 (1933). A New Cutaneous Manifestation in the Syndrome of Vitamin A Deficiency. *Ann. Trop. Med.*, 29, 407 (1935). The Manifestations of A Deficiency in Man.
- McCullough, H., and Dalldorf, G. N., *Arch. Path.*, 24, 486 (1937). Sex Hormones and Vitamin A Requirement.
- Mackie, T. T., and Eddy, W. H., *Am. J. Dig. Dis. and Nutr.*, 6, 617 (1939). Clinical Value of Vitamin Determinations. Unpublished data, 1940. Vitamin A in Cases of Peptic Ulcer.
- Manville, I. A., *Science*, 85, 44 (1937). The Interrelationship of Vitamin A and Glucuronic Acid in Mucine Metabolism.
- Mason, K. E., and Wolfe, J. M., *J. Nutr.*, 9, 725 (1935). Relation of Castration to Vitamin A Deficiency in the Rat.
- Mellanby, E., *Brain*, 54, 247 (1931). The Experimental Production and Prevention of Degeneration in the Spinal Cord. *Brain*, 58, 141 (1935). Lesions of the Central and Peripheral Nervous Systems Produced by A Deficiency.
- Mellanby, E., "Nutrition and Disease," Oliver and Boyd, Edinburgh, 1934.
- Mendel, L. B., *J. Amer. Med. Assn.*, Apr. 23, 1932. Vitamin A.
- Menken, J. G., *Deutsche med Wochnochr.*, 58, 1484 (1932). Blood A Determinations.
- Moore, R. A., and Mack, J., *J. Exper. Med.*, 64, 1 (1936). The Effect of Avitaminosis A on the Prostate.
- Mori, M., *Jahrb. Kinderheil k.*, 59, 175 (1904). Concerning So-called Hikan (Xerosis Conjunctivae).

WHAT ARE THE VITAMINS?

- Mori, S., *Bull. Johns Hopkins Hosp.*, 33, 357 (1922). Changes in Para-ocular Glands Following Diets Low in Fat-Soluble A.
- Nicholls, L., *Indian Med. Guz.*, 68, 681 (1933); 69, 241 (1934). Case Records of A Deficiency.
- Pillat, A., *Archiv. Ophth.*, 2, 399 (1929). Does Keratomalacia Exist in Adults?
- Popper, H., *Proc. Soc. Exp. B. & M.*, 43, 133 (1940). Fluorescent Microscope Determination of Vitamin A.
- Popper, H., and Greengard, Personal Communication, 1940.
- Ralli, E. P., Pariente, A. C., Brandelone, H., and Davidson, S., *J. Amer. Med. Assn.*, 106, 1975 (1936). Effect of Carotene and Vitamin A with Diabetes Mellitus.
- Rowe, E., and Dalldorf, G. N., Unpublished Data, 1937.
- Shibley, G. S., and Spiess, T. D., *J. Amer. Med. Assn.*, 103, 2021 (1934). The Effect of Vitamin A on the Common Cold.
- Spence, J. C., *Arch. dis. Childhood*, 6, 17 (1931). A Clinical Study of Nutritional Xerophthalmia and Night Blindness.
- Steininger, G., Roberts, L. J., and Bergman, S., *J. Amer. Med. Assn.*, 113, 2381 (1939). Vitamin A in the Blood of Normal Adults.
- Stepp, W., and Wendt, G., *Z. f. Exp. Path. and Pharm.*, i, D 31 (1937). Blood Carotene Values.
- Sutton, T. S., Sutterfield, H. E., and Krauss, W. E., *Bull.* 545, Ohio Agric. Exp. Sta., 1934.
- Suzman, M. M., Muller, G. L., and Ungley, C. C. *Am. J. Physiol.*, 101, 529 (1932). An Attempt to Produce Spinal Cord Degeneration in Dogs.
- Wald, G., *J. Gen. Physiol.*, 18, 905 (1935). Vitamin A in Eye Tissues. *Biol. Lab. Symp. on Quant. Biol.*, Cold Spring Harbor, 1935. The Chemistry of the Visual Purple System. *J. Gen. Physiol.*, 19, 781; 20, 45 (1936). Pigments of the Retina.
- Wilson, J. R., and DuBois, R. O., *Am. J. Dis. Chil.*, 26, 431 (1923). Report of a Fatal Case of Keratomalacia in an Infant.
- Wolbach, S. B., and Howe, P. E., *J. Exper. Med.*, 42, 753 (1925). Tissue Changes Following Deprivation of Fat-Soluble A. *Am. J. Pathology*, 9, 275 (1933). The Incisor Teeth of Albino Rats and Guinea Pigs in A Deficiency and Repair. *J. Amer. Med. Assn.*, 108, 7 (1937). The Pathologic Changes Resulting from Vitamin Deficiency.
- Wright, R. E., *Brit. J. Ophth.*, 6, 164 (1922). Keratomalacia in Southern India.
- Youmans, J. B., and Corlette, M. B., *Amer. J. Med. Sci.*, 195, 644 (1938). Specific Dermatoses Due to Vitamin A Deficiency.

VITAMINS A

Yudkin, A. M., Kriss, M., and Smith, A. H., *Am. J. Physiol.*, 97, 611 (1931).
Vitamin A Potency of Retinal Tissue.

Zimmerman, H. M., "Lesions of the Nervous System in Vitamin Deficiency,"
1933.

CHAPTER FOUR

THE PROPERTIES OF VITAMIN B₁ (THIAMINE)

THIS vitamin was first successfully isolated by Jansen and Donath in Java in 1925. R. R. Williams improved the yield by modification of their method, and in 1935 successfully established the chemical character of this vitamin. Cline and Williams (1937) established the accuracy of their chemical structure by synthesizing the product, and synthetic production of the vitamin has now been reported by other laboratories. Many of the properties of B₁ were, however, established before its availability in pure form. By use of B₁ concentrate, Cowgill (1934) showed an important relation of B₁ to calorie intake, and we may well start our discussion of B₁ properties with a review of this basic work.

Loss of appetite, or anorexia, was early associated with the water-soluble B. In 1917 Eddy and Roper successfully used a solution of the vitamin extracted from sheep pancreas to counteract the loss of appetite of infants suffering from malnutrition. In the same year Osborne and Mendel (1917) reported that the food consumption of rats was directly dependent on the amount of B₁ in the diet. Later Karr

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(1920) and Cowgill (1934) demonstrated that the urge of dogs to eat bore a direct relation to their intake of vitamin B.

By contrasting diets containing a high percentage of carbohydrate with diets high in fat or protein, Funk in 1914 showed that the onset of vitamin B₁ deficiency symptoms came much earlier in the case of the high carbohydrate diets. This was the first suggestion that vitamin B₁ needs are related to fuel supply and to the special kind of fuel we call carbohydrate.

Cowgill followed up these findings by working out a mathematical expression for species requirements based on growth response that took the following form:

$$\text{Vitamin B}_1 \text{ need in Int. Units} = K_s \times \text{Wt. in grams}^{5/3}$$

In this formula K_s is a constant varying for different species (s). His values for K_s in this formula are:

$$\begin{aligned} K_s &= .00045 \text{ for the rat} \\ K_s &= .0075 \text{ for the mouse} \\ K_s &= .000185 \text{ for the pigeon} \\ K_s &= .0000038 \text{ for the dog} \\ K_s &= .00000142 \text{ for man} \end{aligned}$$

Obviously, if man's weight is expressed in kilograms instead of grams, K_s becomes .00142, and if expressed in pounds, K_s becomes .00071. The exponent $5/3$ for weight in this formula suggested to Cowgill a possible relation of need to calorie intake. We need not go into the details of this relationship for which the reader is referred to Cowgill's text "Vitamin B Requirements" (Yale University Press, 1934). Suffice it here to say that he showed that his formula could also be written as follows:

$$\text{Vitamin B}_1 \text{ need in Int. Units} = .00142 \times \text{Wt. in Kilograms} \times \text{Calorie intake}$$

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On the basis of this formula, a 70-kilogram man with a calorie intake of 2500 calories would require:

$$.00142 \times 70 \times 2500 = 248 \text{ Int. Units of B}_1 \text{ daily}$$

provided the prediction formula is correct.*

This formula does not give the optimum requirement of vitamin B₁ as Cowgill (1938) himself says:

“It should be emphasized that estimates of the human requirement for vitamin B derived from my formula pertain to the minimum or beriberi preventing level; the optimal intake is undoubtedly much greater.”

This formula of Cowgill's may be expressed in another form. For example:

$$\frac{\text{Vitamin B}_1 \text{ need}}{\text{Calories}} = K_s \times \text{Wt. in Kilograms}$$

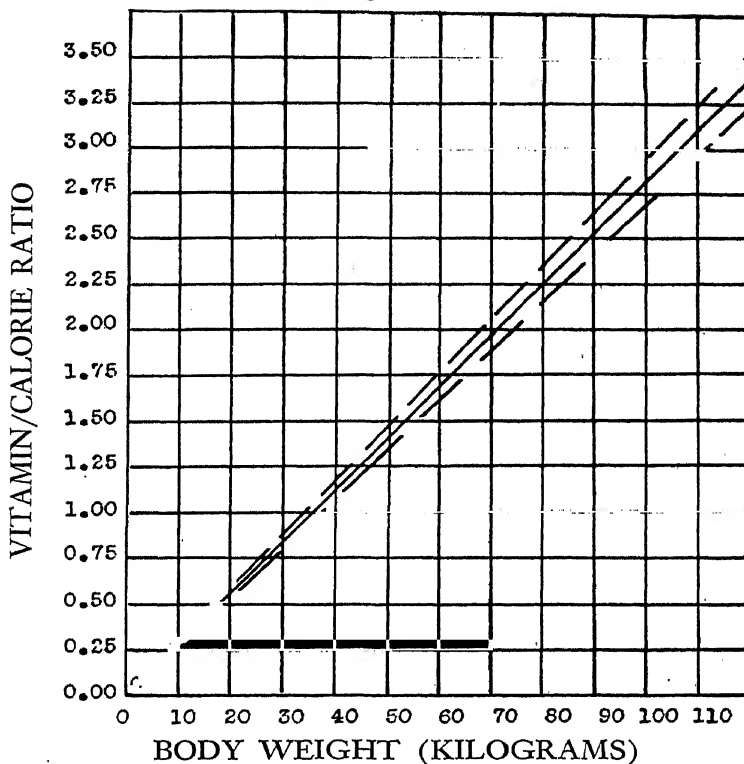
Using this form it is possible to get a vitamin B₁ calorie ratio which is adequate for an individual of a given weight and to use this ratio in estimating vitamin B₁ adequacy of a diet. By applying this formula to a series of diets known to have prevented or failed to have prevented beriberi, Cowgill developed the prediction chart shown on page 63.

With the availability of synthetic vitamin B₁, or thiamine, and the use of the Cowgill formula, progress has been rapid in recent years in establishing that man's need for vitamin B₁ bears a direct relation to his calorie intake and to his ingestion of carbohydrate calories in particular. In the Cowgill formula, the calorie value of the equation is the total calorie intake regardless of whether these calories are

* It is generally agreed that one I.U. of B₁ is equivalent to 3 micrograms of pure thiamine. Multiplying the B₁ need by 3 will therefore give the B₁ requirement in micrograms of thiamine.

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Cowgill's Chart



Prediction Chart (after Cowgill) for estimating the vitamin B₁ adequacy of any diet. The line OA represents the probable minimum vitamin B₁ requirement referred to body weight. If the Vitamin/Calorie ratio of a diet for a given body weight falls above the line OA it is adequate or more than adequate. If it falls below the line OA it is inadequate. (From "Vitamin B Requirement of Man," by G. C. Cowgill, Yale University Press.)

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produced by carbohydrate, fat, protein or other caloric source, such as alcohol.

We have already noted that Funk's experiments indicated that a shift from carbohydrate to fat in the diet reduced vitamin B₁ needs, and this has been confirmed by Evans and associates (1934), by Salmon (1937) and by other investigators. For that reason Williams and Spies (1938) have suggested that only non-fat calories should enter into the prediction formula. Jolliffe (1938) disagreed with this view, and on experimental evidence showed that it made little difference whether he took the vitamin B₁ caloric ratio, the vitamin B₁ carbohydrate caloric ratio, or the vitamin B₁ non-fat caloric ratio. Williams and Spies (1938), following out their contention, suggest the following ratios as borderline for protection of individuals of a given weight:

Table 5.

(After William and Spies, 1938)

Ratio Used	Borderline Value for Protection
Thiamin/total calorie	0.230-0.279
Thiamin/non-fat calorie	1.21 -2.50
Thiamin/carbohydrate calorie	0.251-0.300
Int. Unit/total calorie	1.7 -2.29

Jolliffe has made a correlation between vitamin B₁ caloric ratio and its effect in therapy, and states that improvement in neurological signs of B₁ deficiency do not result when a patient is maintained with a diet containing a B₁ total caloric ratio of 1.7 or less, and that no improvement is produced by adding other members of the B complex when the B₁ caloric ratio is as low as this or lower. However, diets containing a constant amount of B₁ above this ratio, and rich in the entire B complex, apparently lead to a greater improve-

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ment in the objective signs of polyneuritis than diets equally high in B₁ but poor in the B complex, though what fraction of the complex is responsible for this enhanced action of thiamine is not known.

The phosphorylated vitamin B₁, or co-carboxylase, is apparently as effective as the thiamine chloride.

As noted above, the first suggestion that vitamin B₁ was related to carbohydrate metabolism in particular came from Funk (1914). What has transpired since to strengthen this view? This query leads us to another basic study originating in the laboratory of R. A. Peters in Oxford, England.

The Part of Vitamin B₁ in Carbohydrate Metabolism

In 1930 Kinnersley and Peters of Oxford noted a spasm produced in pigeons by an overdose of insulin, and this observation led them to initiate an investigation of the relation of B₁ to brain carbohydrate metabolism in normal and avitaminous pigeons. It was well known that normal brain tissue *in vitro* passed through stages of glycolysis, with the breakdown of the glucose to lactic acid.

When brain tissue of B₁-deficient pigeons was subjected to the same procedure, it was noted that there was an abnormal increase in lactic acid; also that the addition of vitamin B₁ restored tissue respiration in the presence of lactic acid but produced no increase in the absence of the lactate. This led to a search for another intermediate, which was located by Peters and Sinclair (1933) as pyruvic acid.

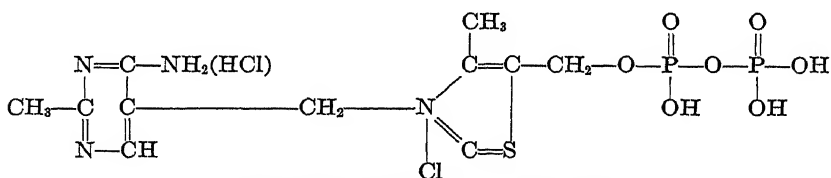
Correlated with these observations of Peters came the demonstration by Platt and Lu (1936) that beriberi cases

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showed an increase of pyruvic acid in the circulating blood; increased blood pyruvate is today used as a diagnostic sign of vitamin B₁ deficiency.

We have explained in Chapter 2 that in conversion of glucose to carbon dioxide and water in tissue cells one of the final steps in the process is the formation of pyruvic acid and its conversion to carbon dioxide and water by oxidation and decarboxylation. The increase in pyruvic acid in the blood of B₁-deficient individuals and its prevention by B₁ dosage therefore suggested that B₁ fitted into the steps of glucose metabolism at the pyruvic acid stage.

A forward step in explanation of how B₁ might act was provided by the isolation from yeast of a coenzyme by Lohman and Schuster (1937). This coenzyme turned out to be a co-carboxylase—in other words, the coenzyme operating with the carboxylase enzyme in splitting carbon dioxide from an organic acid such as pyruvic. Chemical analysis of this co-carboxylase proved it to be the pyrophosphate ester of vitamin B₁ or thiamine. (See also p. 208.)



Co-carboxylase (Thiamin Pyrophosphate)

This discovery made it clear why there is accumulation of pyruvic acid in the brain tissue in the absence of an adequate supply of this co-carboxylase (phosphorylated form of vitamin B₁). Incidentally Ochoa and Peters (1938) report that there is more of the co-carboxylase than thiamine in animal tissue, and that the co-carboxylase can be synthe-

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sized from vitamin B₁ by the liver but not by the intestinal mucosa.

The discovery of co-carboxylase in yeast and its ability to change pyruvic acid to acetaldehyde:



clearly demonstrated that vitamin B₁ in the phosphorylated form played a part in yeast-breakdown of sugar. We are not sure that the breakdown of pyruvic acid takes a similar path in the human tissues; but we are sure that whatever the by-products between pyruvic acid and the ultimate carbon dioxide and water, thiamine pyrophosphate is an essential factor in the transformation, and that inadequate supply of B₁ results in failure to eliminate pyruvic acid and failure to complete the final steps of glucose conversion into energy.

In what way does B₁ inadequacy manifest itself in clinical symptoms? An early viewpoint was that abnormal metabolism in the nerve cell resulted in the accumulation of toxic products which affected the nerve's own functions or the function of tissue in which these toxic products accumulated. Peters' viewpoint is that no such explanation is necessary; that obviously a failure of metabolism of fuel in a tissue is bound of itself to cripple the full efficiency of that tissue. The secondary result will be that regions innervated by nerves so affected will necessarily fail to get normal control, producing results which vary with the tissue or organs so affected. Jolliffe (1938) has summarized diagnostic signs of B₁ deficiency.

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Jolliffe's Signs of B₁ Deficiency

The most definite symptoms are loss of appetite (anorexia) and fatigue, which are non-specific, and also a neurological and circulatory syndrome. Jolliffe cautions that in case of anorexia and fatigue, unless the case shows immediate response to vitamin B₁ therapy, it must be considered that the causes are not B₁ deficiency, since other factors may produce these effects. The neurological manifestations are bilateral and symmetrical polyneuritis involving predominantly the lower extremities. Jolliffe also states that peripheral neuritis, involving a single nerve that is not bilateral or symmetrical or that does not involve the lower extremities, is probably not due to vitamin B₁ deficiency alone.

Heaviness of the lower extremities and calf-muscle cramps are usually the first symptoms. These are followed by alterations of sensitivity (parasthesia) in the toes and fingers, burning of the feet and pain in the legs. When, in addition to these signs, ankle jerks are absent the diagnosis of mild polyneuritis can be made with some definiteness. As the deficiency continues, the sensory and motor changes advance, the knee jerks disappear, position sense in the toes becomes impaired, calf-muscle atrophy develops and foot drop follows. This degree of involvement Jolliffe calls "moderate polyneuritis" provided the signs are confined to the lower extremities. When there is involvement of the upper extremities, spinal cord or cranial nerves or a central neuritis, he classifies the polyneuritis as severe.

The circulatory manifestations of B₁ deficiency, Jolliffe states, may manifest themselves as follows:

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1. Edema and serous effusions in the absence of congestive heart failure, enlarged heart or recognized atrophic factors producing edema and serous effusions.
2. Edema and serous effusions occurring with supporting signs of congestive heart failure and usually evidence of cardiac enlargement.
3. Sudden circulatory collapse which may be the first manifestation of failure or may occur only after other signs of circulatory failure are well advanced.

Some of the characteristic features of circulatory manifestations of vitamin B₁ deficiency are mild polyneuritis, increased or normal blood flow velocity in presence of congestive heart failure, and rapid response to specific B₁ therapy with complete and permanent reversability of the circulatory manifestations.

How does inadequacy of B₁, failure to eliminate pyruvic acid, and increase in carbohydrate or other calories produce these varied symptoms?

Vitamin B₁ and Anorexia

Carlson (1916) has shown that the sensation of hunger is producible by certain rhythmic contractions of the empty stomach. Cowgill and associates (1926) established an association of gastric atony with vitamin B₁ deficiency. Their evidence was secured by means of gastric fistula and fluoroscope and the latter test showed a lowered motility of the entire gastro-intestinal tract.

These observations are in accord with McCarrison's (1921) view that B₁ deficiency results in degeneration of

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the Auerbach's plexus and with Rowland's (1928) observations on gastric atony in B deficiency. In line with these studies it was originally suggested that perhaps the reason anorexia followed B₁ deficiency was because of the lack of the nerve stimulus to these regions.

Today there is little hesitation in accepting gastro-intestinal atony as a sequence to B₁ deficiency, but that it is a sequence to nerve derangement is not so certain. It has been difficult to separate the specific effects of vitamin B₁ deficiency from the effects of inanition. Chattergee (1935), for example, contrasted the intestinal motility in B₁-deficient and in starved animals. In both there was decrease of the amplitude, the number and intensity of intestinal contractions, as well as in the response to pilocarpine, atropine, nicotine and barium chloride. On the other hand, Molitor and Sampson (1938) found that the addition of B₁ to the isolated rabbit intestinal loop produced absolutely no effect on the musculature. Loss of motility, then, may be merely a consequence of poor nutrition, not a lack of some stimulus to muscle activity provided by the vitamin B₁. The problem is still unsolved.

Hypo-acidity

Stepp (1936) reported as a sequence to B₁ deficiency reduced gastro-intestinal motility, anorexia and achlorhydria, or reduced gastric secretion of hydrochloric acid. Babkin (1933) produced definite diminution of response of glands to stimuli while the subject was on a B₁-deficient diet and suggested a relation between B₁ and the nerve complex which controls gastric secretion.

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Strauss (1938) noted similar conditions in B deficiency, but is not sure that some of these results are not due to lack of other factors in the B complex than B₁. Vorhaus (1935), however, has reported correction in eight cases of gastrointestinal hypotonia and anorexia by two months' treatment with 1 to 2 mg. of crystalline B₁ daily.

Peptic Ulcers

Dalldorf and Kellogg (1932) were able to produce chronic ulcers by feeding rats on a B₁-deficient diet. At least, ulcers failed to appear in rats similarly fed except for the vitamin B₁ supplement. These ulcers resembled in every way the chronic ulcers of the human species. McCarrison also found erosion and ulcers of the stomach in his B₁-deficient animals, and it has been suggested that such ulcers perhaps constitute a true anatomical pathology caused by B₁ deficiency.

Dalldorf, however, believes that, as in the case of atony and anorexia, these ulcers are the secondary result of malnutrition rather than a specific effect of lack of B₁. However, since adequacy of B₁ tends to protect against their development, the use of B₁ therapy as an aid in the cure of such conditions is worth consideration, especially since, in the non-irritating diets for ulcer patients, elimination of the irritant matter has tended to make such diets low in vitamin B₁ content.

It may or may not be significant that Cushing (1940) has shown, in certain cerebral operations which were followed by gastric erosion, that the brain areas which control this

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result were the same areas that Peters found affected by B₁ deficiency.

Moore and Plymate (1932) have described a pyloric obstruction in new-born rats whose mothers had been fed a diet low in vitamin B₁, and the curing of this condition by feeding the vitamin. Dalldorf has also suggested that the pyloric thickening observed in elderly persons may be a B₁ deficiency, and justifies special attention to the B₁ content of the diet in planning meals for elderly persons.

Constipation

That vitamin B₁, however it operates, has a definite effect on gastro-intestinal functions, and that it is corrective of certain types of constipation, is the testimony of many clinicians today. Rose and associates (1932) proved that the laxative effect of bran depends on not only its providing bulk but on its B₁ content. Experimentation has shown that the laxative action of mineral oil is enhanced by addition of crystalline vitamin B₁. There is much evidence in the literature of the importance of B₁ as a corrective of gastro-intestinal dysfunction. Marks (1932) found improvement in a large number of cases of what he called simple constipation and colitis by feeding wheat embryo, a rich source of the B complex. Mackie and Pound (1935), from a study of 75 cases of ulcerative colitis, reached the conclusion that relative insufficiency of B₁ might underlie the reduction of tone and the depression of motor function observed in these patients.

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Vitamin B₁ and the Nervous System

Vedder (1938) summarizes existing knowledge regarding beriberi as follows:

“Clinically beriberi is characterized by degenerative changes in the nervous system including a multiple peripheral neuritis, which may exist alone but is often combined with a generalized edema and serous effusions and by a tendency to the development of cardiac hypertrophy which frequently results in cardiac failure and sudden death.”

As we noted previously, acute beriberi is rare in the United States, though common in the Orient. According to Borsook (1938), B deficiency is far from rare and various types of neuritis encountered in clinical practice seem to respond well to vitamin B₁ therapy. This viewpoint has been confirmed by Cowgill (1939).

Strauss (1938) has described the neuro-manifestations of B₁ deficiency in human beings. He states, however, that most patients suffer from partial and irregular deficiency of the vitamin and that months may elapse before marked symptoms occur. Selfridge (1938) claims that the eighth nerve is affected by vitamin B₁ deficiency or some members of the B complex, and has reported good results in five cases of chronic deafness with treatment of vitamin B₁ and corrective dietary.

One of the very striking effects of vitamin B₁ therapy is the promptness of recovery. Just how the B₁ produces this recovery or how it controls the behavior of nerve tissues is still uncertain. If we adopt Peters' view, the nerves suffer loss of function because lack of vitamin B₁ interferes with the

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oxidative metabolism in the nerve tissue; vitamin therapy promptly restores the missing factor.

Vitamin B₁ and Heart Effects

Cardiac failure, rather than neuritis, is the cause of death in human beriberi. On autopsy, the heart of such cases is seen to be markedly dilated and hypertrophied, especially on the right side. The valves are normal and there is no obvious signs of degeneration.

In B₁ deficiency Hastings has observed that the tissue of the auricle in contrast to that of the ventricle shows marked reduction in oxygen uptake from the normal. On this basis Cowgill (1938) has suggested that because of greater susceptibility to vitamin B₁ supply, the auricle becomes weaker, loses tone and as a result suffers greater distention in the presence of pressure exerted by the circulating blood.

In rats, abnormal frequency of heart beat and pulse (bradycardia) has been established to be a consequence of vitamin B₁ deficiency, and measurement of this effect has been successfully employed for the assay of vitamin B₁ in foodstuffs and other B₁ sources (Drury, Harris and Maudsley, 1930). Cowgill states that this effect does not occur in dogs and we have no evidence of its occurrence in man.

Anhydremia

Anhydremia has been noted in the absence of B₁, suggesting possible influence of the vitamin on water metabolism.

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Vitamin B₁ and the Endocrine Glands

In experimental vitamin B deficiency adrenal hypertrophy is rather regularly encountered. Tislowitz (1937) has stated that the parenteral addition of B₁ reduced blood sugar in fasting rabbits and dogs, and Mosonyi and Aszodi (1938) claim that intravenous injection of B₁ increases the secretion of insulin and reduction of blood sugar.

Vorhaus, Williams and Waterman (1935) obtained favorable results in diabetes with the use of B₁ crystals. The question here is whether the vitamin functions in stimulating the secretion of insulin or secures its effect through carbohydrate metabolism or by both methods. Gottlebe (1938) used injections of thiamin chloride in diabetes and claims increased tolerance to sugar, intensification of insulin action and increased secretion of hydrochloric acid in the stomach. The problem needs further investigation with special attention to the effect of B₁ alone in contrast to other members of the B complex.

Lack of B₁ and B₂ has proved detrimental to normal lactation. Sure, 1938 and Evans and Bishop (1922) reported that a reduction in sex functions occurred in rats having a B₁ deficiency.

Vitamin B₁ and Alcoholism

One of the most sensational developments following the availability of pure thiamine for treatment has been the demonstration that primarily the results of excessive alcohol intake are frequently identical with those of vitamin B₁ deficiency. In the light of Cowgill's and Peter's work the explanation is

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clear. A gram of alcohol may yield 7 calories. The alcohol tends as a rule to reduce food intake and increase alcohol calories. The reduced food intake lowers his supply of B_1 while the alcohol calories increase the need for it, and acute B_1 deficiency results. That this is the case has been amply demonstrated by the use of B_1 for treatment of alcoholic states.

Diagnostic Methods

The standard U.S.P. method for demonstrating vitamin B_1 potency of foodstuffs and therapeutic preparations is a bio-assay method. In this method rats are used and are first made polyneuritic by feeding a B_1 -deficient diet. Potencies are determined by the amounts of source necessary to produce recovery in a given period of time in contrast to the effect of pure thiamine.

This method, of course, is entirely too slow for clinical purposes. Some time ago, it was shown that if thiamin is treated with alkali ferricyanide it is converted into the fluorescent substance called thiochrome. The intensity of the fluorescence can be used with a suitable fluorimeter to assay biological fluids. Prebluda and McCollum have developed a colorimetric test which depends upon the reaction between thiamine and a dyestuff. Melnick and Field (1939) have reported successful use of this method in estimating B_1 content of biological fluids such as urine and blood. Schultz, Atkins and Frey (1937) found that the rate of fermentation of yeast is a function of the amount of B_1 present and have worked out a fermentation test for assaying urine content of B_1 . The availability of pure thiamine and the development

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Courtesy Merck & Co., Inc.

Preliminary Chemical Reactions at the Start of a Synthesis

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of rapid clinical tests to determine the effect of vitamin B₁ therapy promise real aid in linking the symptoms we have described to the behavior of the vitamin. That we have need of such diagnostic tests is evidenced by the findings of Cowgill (1939) and others that the American dietary is all too often inadequate in this important vitamin.

To date no adverse results have been reported by the introduction into the body of amounts of B₁ far in excess of the requirement. The minimum requirement of vitamin B₁ for an adult seems to be in the neighborhood of 250 I.U. (750 micrograms of thiamine) and the optimum, 500 to 600 units. When amounts in excess of this are introduced, the excess is rapidly removed, mainly in the urine, and no toxic effects have been observed, with one possible exception. Certain observers claim that with high dosage of B₁ in the absence of adequate choline, there may be a tendency toward fatty infiltration of the liver. Choline appears to prevent such a tendency.

In the selection and preparation of vitamin B₁ food sources for the table it should be borne in mind that although this vitamin is not affected by oxidation it can be destroyed by heat if exposed to it for a sufficient length of time. The table from Elvehjem (1939) (p. 79) shows the extent of destruction of vitamin B₁ in the cooking of meats.

Melnick and Field (1940) have also recently shown that if the ingestion of vitamin B₁ is preceded by taking of antacids there is an appreciable destruction of vitamin B₁ in the gastrointestinal tract; and we know that the addition of soda in the cooking of vegetables has a definitely destructive effect on this particular vitamin. (For its distribution in common foodstuffs see Appendix, pages 219 seq.)

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Table 6.
(After Elvehjem, 1939)

Source of B ₁ and Method of Cooking	Percent Destruction B ₁
Beef round, roasted	61
Beef round, broiled	60
Veal quarter, fried	45
Veal quarter, roasted	58
Pork loin, fried	35
Pork loin, roasted	50
Pork ham, fried	0
Pork ham, smoked	10
Beef heart, stewed one hour	55
Beef kidney, stewed one hour	44

Bibliography

- Babkin, B. P., *Canad. Med. Assn. J.*, 29, 5 (1933). Nervous Control of Gastric Secretion and Effect of Vitamin Deficiency on Its Production.
- Borsook, H., Dougherty, P., Gould, A. A., and Kremers, E. D., *Am. J. Dig. Dis. and Nutr.*, p. 246 (1937). Value of B Complex in Constipation Cases.
- Carlson, A. J., "The Control of Hunger in Health and Disease," University of Chicago Press, 1916.
- Chattergee, D. D., *Indian J. Med. Res.*, 23, 191 (1935). The Motor Function of the Bowel in Avitaminosis B.
- Cline, J. K., Williams, R. R., and Finkelstein, J., *J. Am. Chem. Soc.*, 59, 1052 (1937). Synthesis of Vitamin B₁.
- Cowgill, G. R., "The Human Requirement for Vitamin B." Yale University Press, 1934.
- Cowgill, G. R., *J. Am. Med. Assn.*, 110, 805 (1938). The Physiology of Vitamin B₁. *J. Am. Med. Assn.*, 111, 1009 (1938). Human Requirements for Vitamin B₁. *J. Am. Med. Assn.*, 113, 2146 (1939). The Need for the Addition of Vitamin B₁ to Staple American Foods.
- Cowgill, G. R., Deuel, H. J., Jr., Plimmer, N., and Messer, F. C., *Am. J. Physiol.*, 77, 389 (1926). Vitamin B in Relation to Gastric Motility.
- Cushing, H., Cited by Dalldorf.
- Dalldorf, G. N., and Kellogg, M., *J. Exper. Med.*, 56, 391 (1932). Incidence of Gastric Ulcer in Albino Rats Fed Diet Deficient in B₁.

WHAT ARE THE VITAMINS?

- Drury, A. N., Harris, L. J., and Maudsley, C., *Biochem. J.*, **24**, 1632 (1930). Vitamin B Deficiency in the Rat; Bradycardia as a Destructive Feature.
- Eddy, W. H., and Roper, J. C., *Am. J. Dis. Child.*, **14**, 189 (1917). The Use of the Pancreatic Vitamin in Cases of Marasmus.
- Elvehjem, C. A., Personal Communication. Cooking Effect on B₁.
- Evans, H. M., and Bishop, K. S., *Am. J. Physiol.*, **63**, 396 (1922). B₁ and Sex Functions.
- Evans, H. M., Lepkovsky, S., and Murphy, E. A., *J. Biol. Chem.*, **107**, 429 (1934). The Sparing Action of Fat on Vitamin B.
- Funk, C., *Ztschr. f. physiol. Chem.*, **89**, 378 (1914). The Role of Vitamine in Carbohydrate Metabolism.
- Funk, C., and von Schönborn, E., *J. Physiol.*, **48**, 328 (1914). The Influence of a Vitamin-Free Diet on Carbohydrate Metabolism.
- Gottlebe, *Ztschr. Klin. med. Berlin*, **133**, 739 (1938). Vitamin B₁ and Carbohydrate.
- Jansen, B. C. P., and Donath, W. F., *Mededeel. Dienst. Volksgezondheid, Nederland Indie*, Pt. 1, p. 186 (1926). *Nature*, **135**, 267 (1935). Isolation of Vitamin B₁.
- Jolliffe, N., *Int. Clinics*, Series 48, p. 47. Recognition of Vitamin B₁ Disease in America.
- Jolliffe, N., and Colbert, C. N., *J. Amer. Med. Assn.*, **107**, 642 (1936). The Etiology of Polyneuritis in the Alcohol Addict.
- Jolliffe, N., and Goodhart, R., *J. Amer. Med. Assn.*, **111**, 380 (1938). Beri-beri in Alcohol Addicts.
- Karr, W. G., *J. Biol. Chem.*, **44**, 255 (1920). Some Effects of Water-Soluble Vitamine Upon Nutrition.
- Kinnersley, H. W., and Peters, R. A., *Biochem. J.*, **24**, 1856 (1930). Opisthotonos with Insulin in B₁ Deficiency.
- Lohman, K., and Schuster, P., *Naturwissensch.*, **25**, 26 (1937). Concerning Co-carboxylase.
- McCarrison, R., "Studies in Deficiency Diseases," London, 1921.
- McHenry, S. W., *Biochem. J.*, **31**, 1616 (1937). An Effect of Choline on the Vitamin B₁ Sparing Action of Fats.
- Mackie, T. T., and Pound, R. E., *J. Amer. Med. Assn.*, **104**, 613 (1935). Changes in the Gastro-Intestinal Tract in Deficiency States.
- Marks, H. A., *Medical Record*, March 2, 1932. Vitamin B Deficiency.

VITAMIN B₁

- Melnick, D., Field, H., and Prebluda, W. D., *J. Nutr.*, **18**, 593 (1939); *J. Biol. Chem.*, **127**, 505, 515, 531 (1939). Quantitative Study of Urinary Excretion of Thiamine and Prebluda Method.
- See also: Emmett, A. D., Peacock, G., and Brown, R. A., *J. Biol. Chem.*, **135**, 131 (1940). Chemical Determination of Thiamine by a Modification of the Melnick-Field Method.
- Molitor, H., and Sampson, W. L., Personal Communication, 1938.
- Moore, C. U., and Plymate, H. B., *Am. J. Physiol.*, **102**, 605 (1932). Studies on the B Vitamin; Further Consideration of Pyloric Obstruction in Rats.
- Mosonyi, J., and Aszodi, Z., *Klin. Wochenschr.*, **17**, 337 (1938). Vitamin B and C and Vagus Stimulation.
- Ochoa, S., Peters, R. A., and Stocken, L. A., *Nature*, **144**, 750 (1939). *Biochem. J.*, **33**, 1262 (1939). Pyruvic Acid Metabolism in the Brain.
- Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **31**, 149 (1917). The Role of Vitamins in the Diet.
- Passmore, R., Peters, R. A., and Sinclair, H. M., *Biochem. J.*, **27**, 842 (1933). Oxygen Uptake of Avitaminous Brain.
- Peters, R. A., *Current Sci.*, **5**, 207 (1936). Review of Vitamin B₁ Functions.
- Platt, B. S., and Lu, G. D., *Quart. J. Med.*, **5**, 355 (1936). Chemical and Clinical Findings in Beri-beri with Special Reference to Vitamin B₁ Deficiency.
- Prebluda, H. P., and McCollum, E. V., *Science*, **84**, 488 (1936). A Chemical Reagent for the Detection and Estimation of Vitamin B₁.
- Rose, M. S., McLeod, G., Vahlteich, E. C., Funnell, E. H., and Newton, C. L., *J. Am. Dietetic Assn.*, **8**, 133 (1932). The Influence of Bran on the Alimentary Tract.
- Salmon, W. D., and Goodman, J. G., *J. Nutr.*, **13**, 477 (1937). Alleviation of Vitamin B Deficiency in the Rat by Certain Natural Fats and Synthetic Esters.
- Schultz, A., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, **59**, 948, 2457 (1937); **60**, 1514 (1938). A Fermentation Test for Vitamin B₁.
- Selfridge, G., *Annal. Otolaryngology, Rhinology, Laryngology*, **46**, 93 (1937). Eighth Nerve Tone Deafness from a Nutritional Standpoint.
- Spies, T. D., and Aring, C. D., *J. Amer. Med. Assn.*, **110**, 1081 (1938). Effect of Vitamin B₁ on the Peripheral Neuritis of Pellagra.
- Stepp, W., "Die Vitamine," Stuttgart, Enke, 1936.
- Strauss, M. B., *J. Amer. Med. Assn.*, **110**, 953 (1938). The Therapeutic Use of Vitamin B₁ in Polyneuritis and Cardiovascular Conditions.

WHAT ARE THE VITAMINS?

- Tislowitz, R., *Klin. Wochenschr.*, **16**, 226 (1937). Vitamin B₁ and Carbohydrate Metabolism.
- Vedder, E. B., *J. Am. Med. Assn.*, **110**, 893 (1938). The Pathology of Beri-beri.
- Vorhaus, M. G., Williams, R. R., and Waterman, R. E., *Am. J. Digest. Dis. and Nutr.*, **2**, 541 (1935). Studies on Crystalline Vitamin B₁.
- Williams, R. R., and Spies, T. D., "Vitamin B₁" (Text). Macmillan, 1938.
- Williams, R. R., Waterman, R. E., and Keresztesy, J. C., *J. Am. Chem. Soc.*, **56**, 1187 (1934). Larger Yields of Crystalline Antineuritic Vitamin.

CHAPTER FIVE

THE FUNCTION OF RIBOFLAVIN (VITAMIN B₂ or G)

THE viewpoint that McCollum and Kennedy's (1916) water-soluble B contained more than one vitamin was first suggested by Mitchell (1919); and in 1920, Emmett and Luros (1920) proved that, by a heat treatment, they could destroy completely the antineuritic action of yeast without destroying its growth-promoting action on rats. This early work demonstrated that there were present in water-soluble B at least two water-soluble vitamins—one corrective of polyneuritis and heat-labile, the other growth-promoting, heat-stable, and of no effect on polyneuritis.

Goldberger (1925, 1926) suggested that the heat-stable factor might be concerned in the cure of pellagra. At the time he suggested the following names:

Vitamin A-N for the heat-labile, antineuritic vitamin.

Vitamin P-P for the heat-stable, pellagra-preventive vitamin.

In England and on the continent the terms "B₁" for the antineuritic factor and "B₂" for the heat-stable factor were later adopted, and in America the heat-stable factor was designated as vitamin G.

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By 1931 a quantitative method for estimating the relative amounts of vitamins B and G had been worked out in Dr. Sherman's laboratory; B₁ assay by Chase and Sherman (1931) and G assay by Bourquin and Sherman (1931).

Further study of the character of water-soluble vitamin B, however, soon showed that the substance which was revealed by the Bourquin-Sherman test was not pellagra-preventive, though it was a growth-promoting factor and a preventive of a certain type of dermatitis. This discovery initiated the fractionation of water-soluble B that has gone on actively ever since and which, as shown on p. 5, has revealed to date thirteen or fourteen possible vitamins in this fraction.

Meanwhile the vitamin G or B₂ was identified as a sugar-dye combination to which at present we give the name of *riboflavin*, because the sugar in the molecule is ribose. This substance was demonstrated in various products always showing a flavin nucleus. Such discoveries led to its earlier designation by source, namely, lacto-flavin, hepato-flavin, etc. The proof that all these substances were the same product led later to the rejection of these separate names and universal adoption of riboflavin as its designation (see Appendix, page 208).

Riboflavin has been known for some years. Winter Blyth first noted it, and it was described by Bleyer and Kallman (1925) under the name of lactoflavin in 1925. None of these investigators suggested any biologic significance or explanation of its chemical nature.

In 1932 Warburg and Christian described a new oxidation enzyme derived from yeast, which was yellow in water solution and had a greenish fluorescence. They called it the yellow enzyme (see Chapter 3). In 1933 Booher in Sher-

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man's laboratory and Kuhn, P. György and Wagner-Jauregg (1933) on the Continent demonstrated that a yellow pigment extracted from whey and egg would serve as a growth-promoting supplement when added to the Bourquin-Sherman vitamin G-free basal diet. This pigment was called ovoflavin when derived from egg, and lactoflavin when derived from milk; it was shown by Kuhn and co-workers to be related to Warburg's yellow enzyme. We know now that riboflavin is the prosthetic or active part of the Warburg yellow oxidation ferment, and this fact as we have explained in Chapter 3, shows its importance in biological oxidations.

While this identification of vitamin G's chemical nature was developing, progress was also made toward defining the type of dermatitis produced in rats on a G-deficient diet. The steps in this study have been reviewed by Hogan (1938) who also contributed significantly to their elucidation. Unlike the lesions of human pellagra or of blacktongue in dogs, riboflavin deficiency in rats is characterized by a bilaterally symmetrical denudation or loss of hair. There is atrophy of the sebaceous glands, thinning of the epithelium, and hyalination of the tail (Smith and Sprunt, 1935).

As a result of such studies we have today considerable knowledge concerning the effect of riboflavin deficiency on rats. We know that it has a non-specific effect on growth and that negatively it does not correct the specific lesions of human pellagra or blacktongue in dogs, and is therefore not what Goldberger designated as the P-P vitamin.

Human Needs for Riboflavin

Goldberger's P-P vitamin later turned out to be nicotinic

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acid (see Chapter Six). Spies and co-workers (1938, 1939) showed that riboflavin and vitamin B₁, in addition to nicotinic acid, were frequently necessary to correct collateral deficiencies of pellagrins; in other words, that in the ordinary pellagrin the disease manifestations were not purely those of nicotinic acid deficiency.

Studying these collateral deficiencies still further, Sebrell and Butler (1938) reported that specific lesions occurring in the corners of the lips and previously recognized under the name of cheilitis or cheilosis were apparently a *specific* effect of riboflavin deficiency. Using a diet composed of corn meal, cow peas, lard, casein, white flour, white bread, calcium carbonate, tomato juice, cod liver oil, syrup and iodide of iron, Sebrell and Butler (1938) produced, in ten out of eighteen women, a pallor of the mucosa in the angles of the mouth. These areas became macerated and in a few days transverse superficial cracks or fissures appeared. The lesions remained moist and were covered with a honey-colored crust which could be removed without causing bleeding. The condition was similar to that previously noted in children and characterized as perleche. These lesions did not respond to treatment with nicotinic acid, but did respond to treatment with riboflavin in 5-mg. doses.

Sydenstricker and associates (1939) have confirmed Sebrell and Butler's claim that cheilosis responds to riboflavin therapy. This is the first specific effect of riboflavin deficiency in human beings reported to date.

Day (1931) reported that riboflavin deficiency produced cataract in rats. Between 1934 and 1937 he reported ability to duplicate this effect in mice, chickens and monkeys and

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to show that synthetic riboflavin would cure the condition, making this substance apparently a specific factor.

In 1935 Mitchell and Dodge produced a type of cataract by galactose feeding. Morgan and Cook (1936) showed that riboflavin did not correct this form of cataract. These results indicate that there may be different factors involved in the production of cataract, and that for some of these riboflavin is certainly not a specific corrective.

Bessey and Wolbach (1938) noted that in riboflavin deficiency in the rat there was infiltration of blood vessels into the cornea of the eye, and stated that this phenomenon preceded all other demonstrable lesions of the deficiency. In other words, somewhat earlier than the appearance of skin lesions in vitamin B₂ deficiency, capillaries began to grow into the cornea, and within three months extended one-third of the way across the cornea, some reaching its center. The vessels were in the form of a netted plexus which lay immediately beneath the epithelium, but which later invaded the deeper structures. These lesions were rapidly reversed by treatment with riboflavin. Eckhart and Johnson (1939) have also reported corneal vascularization in riboflavin deficiency. Only two out of twelve of their rats, however, actually developed cataract.

Eckhart and Johnson say that the galactose cataract is not associated with vascularization, and agree with Morgan and Cook (1936) that it is not cured by riboflavin. Day claims that the failure of some investigators to duplicate his findings is due to their failure to use a completely riboflavin-deficient diet. The matter is therefore in need of further study before we can be sure that riboflavin is a significant factor in the cure or prevention of human cataract.

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Castle suggested a possible role of riboflavin in anemia prevention. His views have been reviewed by Rhoads (1939). It has been known for some time that the pernicious anemia-preventive factor first described by Minot and Murphy (1926) as occurring in liver is produced by the interaction of two factors in the stomach. The gastric secretion supplies one of these factors and a second one must be present in the diet. When these two factors unite, the anti-anemic substance is formed and transferred for storage to the liver. The factor provided in the diet is called the *extrinsic factor* and that supplied by the secretion, the *intrinsic factor*. It was suggested that the extrinsic factor might be riboflavin.

Castle performed the following experiment. He ate beef muscle, allowed it to digest in his own stomach, then removed, neutralized it, fed the mixture to pernicious-anemia subjects, and effected a cure in these cases. He proved in a controlled experiment that the beef muscle alone, before this stomach digestion, was not effective as a cure; that the gastric juice from the stomach alone was also not effective; but that, when the two were incubated together, a protective substance was formed. Further study of this intrinsic factor demonstrated its presence in yeast, muscle, liver, eggs, malt extract, barley, wheat germ, and rice bran, all of which, of course, are excellent sources of B complex and riboflavin.

The suggestion was, then, that the riboflavin might be Castle's extrinsic factor. Ashford and associates (1936) were unable to produce the extrinsic factor by incubating pure riboflavin with normal gastric juice or the press juice of hog's stomach. According to Castle, the extrinsic factor is water-soluble, soluble in 80% alcohol and acetone, and heat-stable even in alkaline solution. Reimann (1936) expresses doubt

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of its identity with riboflavin; but again the problem is not yet solved.

P. György, Robbins and Whipple (1938) have reported that in standardized anemic dogs, daily doses of 0.1 to 0.5 mg. of riboflavin per kilogram of body weight caused definite increase in hemoglobin formation; the rise was approximately one-quarter the effect of 300 gm. of pig liver. Others have also indicated that it is a potential factor in nutritional anemia.

Riboflavin and Tissue Respiration

As we have shown in Chapter Two, Warburg's yellow enzyme is a combination of riboflavin with phosphoric acid and a protein. The protein is supposed to supply the adsorbing surface which is specific for binding certain compounds and bringing them into contact with the prosthetic group; that when such compounds are adsorbed on the protein surface the phosphorylated riboflavin is able to function as a hydrogen acceptor and thus as a carrier of hydrogen in the series of metabolic oxidation changes. By change in the protein and other modifications, it is possible for riboflavin to be a constituent of several enzymes with different specificity, and several such have been already isolated and identified. (See Chapter Two.)

That riboflavin is an essential component of the prosthetic group in cell oxidation indicates that it is important in cell respiration, though the daily human requirement to supply adequacy for this purpose is not yet known. Two milligrams per day has been suggested as the minimum need.

Adams (1936) showed that when rats received a diet low

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in riboflavin the oxygen uptake of their skin was definitely lowered, supporting its relation to intracellular respiration and metabolism. The yellow enzymes have been shown to be widely distributed in body tissues, and this fact alone would support a claim of importance in dietary supply of this factor to insure ordinary tissue respiration and metabolism.

Cytoflav

It is now generally held that before riboflavin can be built into the yellow enzyme and function as hydrogen carrier it must be phosphorylated. Iodoacetic acid retards such ester formation. Laszt and Verzar (1935) retarded growth and produced hypertrophic adrenals and alterations in the bones, skin and blood of rats by feeding a complete diet to which had been added 0.02% iodoacetic acid. The addition of pure riboflavin to this diet did not restore growth, but 0.02% of phosphorylated riboflavin accomplished this effect. Szent Gyorgyi has called the phosphorylated riboflavin "cytoflav".

Quantitative Needs for Riboflavin

Riboflavin occurs in plants and animals in at least three forms: as the free flavin, in combination with phosphate, and in combination with protein and phosphorus as yellow enzymes.

Sherman and Langford (1938) reviewed human requirements for riboflavin. They pointed out that, especially with rats, deficiency stunted the growth of the young and caused lowering of general tone and a condition of premature aging

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of the skin, with loss of hair. They also reported that the optimum needs for the rat appeared to be several times that necessary to prevent visible signs of the deficiency, but they did not give any experimental evidence as to the quantity needed by adults.

Emmerie (1936, 1937) reported that the daily urinary excretion of male subjects was 30-50 micrograms per hour, but that the output was increased with increase of the intake. In general, however, his findings suggest that a daily intake of from 2 to 3 mg. of riboflavin (666 to 1000 Sherman-Bourquin units) should compensate for normal excretion and keep reserves normal in a 140-lb. adult, though there is apparently some destruction of riboflavin in the body.

Stiebeling and Phippard (1939), in Bulletin 507 of the U. S. Department of Agriculture, suggest 600 Sherman-Bourquin units or 1800 micrograms (1.8 mg.) of riboflavin daily as desirable human adult allowance.

The dosage used by Sebrell and Butler in treating cheilosis cases was 1-2 mg. for 3 to 10 days and then 0.025 mg. per kilogram of body weight daily. Sydenstricker *et al.* (1939) used 10 mg. in 200 cc. of physiological salt solution daily.

There were reports at one time that large doses of riboflavin produced toxic effects, but the evidence now appears to show that the effect of such dosages was due to the solvent used and not to the riboflavin. In tests on animals, amounts 5000 times the minimum requirement to prevent symptoms have been used without any toxic effects.

Fortunately, riboflavin is quite widely distributed in natural foodstuffs and consequently there is not grave danger of deficiency of this factor in the ordinary diet. (For such distribution see Appendix, page 218 seq.)

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Other Suggested Uses of Riboflavin

Lepkovsky and Jukes (1935, 1936) showed that riboflavin is essential for the growth of the chick, and P. György (1938) showed that chronic riboflavin deficiency resulted in pediculosis (louse production).

Basu (1938) claims that B₂ is a definite factor in the development of leprosy and that deficiency of it lowers resistance to endemic typhus. We have already noted that Maitra (1937) claims that riboflavin deficiency is definitely productive of achlorhydria or reduced gastric acidity. It is now available in crystalline form and in concentrates and its fluorescent property has made possible clinical tests on blood and urine content.

Bibliography

- Adams, P. D., *J. Biol. Chem.*, **116**, 641 (1936). Effect of Vitamin G on Skin Respiration.
- Ashford, C. A., Klein, L., and Wilkinson, J. F., *Biochem. J.*, **30**, 218 (1936). Note on Non-identity of Lactoflavin and Extrinsic Factor.
- Basu, N. K., *Z. Vitaminsforsch.*, **3**, 194 (1934). B₂ Deficiency Lowers Resistance to Leprosy.
- Bleyer, B., and Kallman, O., *Biochem. Ztschr.*, **155**, 54 (1925). Concerning a Little-studied Constituent of Milk.
- Blyth, A. Winter, *J. Chem. Soc.*, **35**, 530 (1879). The Composition of Cow's Milk in Health and Disease.
- Booher, L. E., *J. Amer. Med. Assn.*, **110**, 1105 (1938). *J. Biol. Chem.*, **102**, 39 (1933); **119**, 223 (1937). The Chemistry of Riboflavin.
- Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, **52**, 3501 (1931). Quantitative Determination of B₂ (G).
- Chase, E. F., and Sherman, H. C., *J. Am. Chem. Soc.*, **53**, 3506 (1931). The Quantitative Determination of Vitamin B₂.

VITAMIN B₂ OR G

- Day, P. L., Langston, W. C., and O'Brien, C. S., *Am. J. Ophth.*, 14, 1005 (1931); also, *J. Nutr.*, 7, 97 (1934); 13, 389 (1937). Vitamin G Deficiency and Cataract.
- Eckhart, R. E., and Johnson, L. V., *Arch. Ophth.*, 21, 315 (1929). Nutritional Cataract and Relation of Galactose.
- Emmerie, A., *Nature*, 138, 164 (1936); *Acta brev. Nederlund*, 7, Nos. 4 and 5 (1937). Excretion of Flavins in Normal Human Urine.
- Emmett, A. D., and Luros, G. O., *J. Biol. Chem.*, 43, 265 (1920). Are the Antineuritic and the Growth-Promoting Water-soluble Vitamins the Same?
- Goldberger, J., and Tanner, W. F., *Pub. Health Rep.*, 40, 58 (1925). A Study of Pellagra Prevention by Certain Foods.
- György, P., *Proc. Soc. Exp. B. & M.*, 38, 385 (1938). Production of Pediculosis in Vitamin G Deficiency.
- György, P., Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 122, 154 (1938). Lactoflavin Increases Hemoglobin Production in Anemic Dogs.
- Hogan, A. G., *J. Amer. Med. Assn.*, 110:1105 (1938). Riboflavin: Physiology and Pathology.
- Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber. d. deutschen chem. Gesellsch.*, 66, 1034 (1933). Concerning Lactoflavin.
- Kuhn, R., Rudy, H., and Wagner-Jauregg, T., *Ber. d. deutschen chem. Gesellsch.*, 66, 1950 (1933). Concerning Lactoflavin.
- Laszt, L., and Verzar, F., *f.d. Arch. ges. Physiol. (Pflugers)*, 236, 693 (1935). Antagonism between Vitamin G and Iodoacetic Acid.
- Lepkovsky, S., and Jukes, T. H., *J. Nutr.*, 12, 515 (1936). The Response of Rats, Chicks, Turkey Poults, to Crystalline Vitamin G.
- McCollum, E. V., and Kennedy, C., *J. Biol. Chem.*, 24, 491 (1916). The Dietary Factors Operating in the Production of Polyneuritis.
- Maitra, K., *Biochem. J.*, 31, 2322 (1937). Relation between B₂ Deficiency and Achlorhydria.
- Minot, G. R., and Murphy, W. P., *J. Amer. Med. Assn.*, 87, 470 (1926). Treatment of Pernicious Anemia by a Special Diet.
- Mitchell, H. H., *J. Biol. Chem.*, 40, 399 (1919). On the Identity of the Water-Soluble Growth-Promoting Vitamin and the Antineuritic Vitamin.
- Mitchell, H. S., and Dodge, W. M., *J. Nutr.*, 9, 37 (1935). Cataract in Rats Fed on High Lactose Rations.

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- Morgan, A. F., and Cook, B. B., *Proc. Soc. Exp. B. & M.*, 34, 281 (1936). Cataract and Dermatitis Producing Nutritional Factor.
- Reimann, F., *Med. Klin.*, 27, 880 (1931). Vitamin G Probably Not the Extrinsic Factor. See also: Reimann, F., and Weil, R., *Ztschr. f. klin. Med.*, 126, 568 (1934).
- Rhoads, C. P., *J. Amer. Med. Assn.*, 113, 297 (1939). Conference on Therapy Vitamin B₂.
- Sebrell, W. H., and Butler, R. E., *J. Am. Med. Assn.*, 111, 2286 (1938). Vitamin G Deficiency and Cheilosis.
- Sherman, H. C., and Langford, C. S., *J. Amer. Med. Assn.*, 110, 1278 (1938). Riboflavin; Dietary Sources and Requirements.
- Smith, S. G., and Sprunt, D. H., *J. Nutr.*, 10, 481 (1935). Pathological Skin Changes in the Tail of the Albino Rat on a Diet Deficient in Vitamin G.
- Spies, T. D., Vilter, R. W., and Ashe, W. F., *J. Amer. Med. Assn.*, 113, 931 (1939). Pellagra, Beri-beri, and Riboflavin Deficiency in Human Beings.
- Stiebeling, H., and Phippard, E. F., Bulletin 507, U. S. Dept. Agriculture, 1939.
- Strauss, M. B., and Castle, W. B., *Lancet*, 2, 111 (1932). Extrinsic Factor in Pernicious and Related Anemias.
- Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, D., *J. Amer. Med. Assn.*, 114, 2437 (1940). The Ocular Manifestations of Ariboflavinosis.
- Theorell, H., *Biochem. Z.*, 272, 37, 155, 275 (1934); 278, 263 (1935). Crystallization and Structure of Respiration Ferment.
- Warburg, O., and Christian, W., *Biochem. Ztschr.*, 254, 438 (1932). A New Oxidation Ferment and Its Absorption Spectrum.

CHAPTER SIX

THE FUNCTIONS OF NICOTINIC ACID (VITAMIN P-P)

AFTER Casimir Funk had isolated from rice polishings the beriberi-curative substance which he called "vitamine", this substance was shown to contain nicotinic acid; and since nicotinic acid had no effect on beriberi it got no position in the vitamin group. In 1937, Funk suggested that nicotinic acid supplemented the growth effect of thiamine, but further study of this effect showed it to be relatively unimportant (Frost and Elvehjem, 1937).

In the same year, however, Elvehjem, Madden, Strong, and Wooley (1937) reported that they had separated from liver extract a substance curative of blacktongue in dogs, and that the significant constituent of this liver fraction was nicotinic acid or amide. Blacktongue in dogs is believed to be similar to human pellagra in cause and symptoms. In Chapter 4 we noted that Goldberger postulated a pellagra-preventive factor in the water-soluble B complex, which was first believed to be the heat-stable part identified as B₂, G, or riboflavin. Failure of this substance to cure either blacktongue or pellagra initiated further search for Goldberger's P-P factor; and the discovery of Elvehjem *et al.*

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(1937) that the curative liver fraction contained nicotinic acid or amide suggested that the search was ended.

Proof of this was soon forthcoming. (Fouts, 1937; Smith, 1937; Harris, 1937; France, 1937; Spies *et al.*, 1938; Sebrell, 1938.) Nicotinic acid, nicotinic acid amide, sodium nicotinate, and to a lesser degree coramine or diethyl amide have all been shown to cure the glossitis and stomatitis of typical pellagra.

Spies (1939) describes pellagra as a syndrome affecting the skin, alimentary tract and the central nervous system. Diagnosis is made by observing characteristic glossitis (tongue inflammation), characteristic dermatitis (skin inflammations) or both. The skin is roughened, reddened, scaling, cracked and sharply differentiated from normal skin. These lesions are bilaterally symmetrical, appearing most frequently on the back of the hands, the elbows, knees, ankles, neck, and underarm regions. The lesions of the alimentary tract are usually first to appear. There is loss of appetite, burning of the tongue giving way to intense tongue, mouth, gum and pharyngeal inflammation. Later stomach and intestinal inflammations follow. There is often reduced gastric secretion and severe diarrhea, with abdominal pain and distention. There also may be severe vaginitis and urethritis. The central nervous changes may culminate in paranoid delusions and hallucinations. The disease usually appears in late spring and early summer and is aggravated by exposure to sunlight. Pellagrins usually show the presence of the pigment porphyrin in excess in the urine (porphyrinuria). It has been suggested that the presence of the porphyrins in body regions may account for photosensitivity in those regions, but this has not been substantiated.

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Goldberger and his associates (1932) clearly demonstrated that pellagra was producible by specific diets and that inclusion of certain foods in the diet would prevent its occurrence and cure the disease. Walker and Wheeler (1931) produced the disease by restricting the diet to corn meal, black-eyed peas, lard, flour, cane sirup, white bread, cod liver oil and tomato juice, and Goldberger and Sebrell (1933) recommended the following dietary treatment of pellagra:

“A food intake of 3000 calories per day should be the aim (in mild cases correction of the diet is all that is needed). Milk should be the principal item of the diet; beef juice or meat soups and broths in small quantities at frequent intervals in gradually increasing amounts; solid food, particularly fresh lean meat and liver as soon as the patient's digestive system will permit; pure dried yeast in one-half to one ounce dosage daily in milk, tomato juice or table sirup and liver extracts in liberal doses in difficult cases. It should be particularly emphasized that . . . success in treatment of the individual cases will be in almost direct proportion to the attention devoted to the proper feeding of the patient.”

The discovery of nicotinic acid in the curative liver extract explained why certain foods had beneficial effect; for we know now that their preventive or curative value is directly proportional to their nicotinic acid or amide content. Using the cure of black-tongue in dogs as an assay method in contrast to nicotinic acid as a reference standard, Elvehjem (1939-40) has given the distribution in certain foods shown in Table 7.

Elvehjem (1939-40) obtained these results by use of dogs on a black-tongue producing diet consisting of: 72 parts yellow corn, 18 parts purified casein, 5 parts cotton seed oil,

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Table 7. Nicotinic Acid Content of Foods.

(After Elvehjem)

Foodstuff	Mg. Nicotinic Acid per gm. of dry material	Foodstuff	Mg. Nicotinic Acid per gm. of dry material
Liver, pork	1.2	Brain, beef	0.3-0.5
Liver, lamb	1.2	Heart, pork	0.3
Liver, veal	0.9	Heart, beef	0.3
Kidney, pork	0.85-1.0	Yeast, brewer's	1.0
Pork loin	0.45-0.6	Yeast, baker's	0.5
Pork ham	0.4	Skim milk powder	0.05-0.15
Beef tongue	0.4-0.5	Wheat germ	0.05-0.10
Veal	0.5	Dried cereal grass	0.10-0.15

2 parts of cod liver oil, one part each of $\text{Ca}_2(\text{PO}_4)_2$, CaCO_3 , and NaCl ; 50 micrograms each of thiamin and riboflavin per dog per day.

Harris and Raymond (1939) and others have reported chemical methods for assay of nicotinic acid in foodstuffs. Harris *et al.* claim their test sensitive to .001 mg. of the acid. Its use should extend our knowledge of nicotinic acid distribution and eliminate the more time-consuming bio-assay methods. Meanwhile, thanks to Sebrell's studies of foods in actual treatment of pellagrins, we have the list given in Table 8.

Nicotinic Acid Therapy

Spies and associates (1938, 1939) have recommended for severe cases 500 mgm. nicotinic acid daily in five 100-mg. doses, though cures have been effected with as little as 60-70 mg. daily (Ruffin, 1939). In one series Spies, Bean and Stone (1938) reported that a group of children afflicted with the disease responded to dosage as follows:

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5 % got relief with 50 mg. daily orally.
 50 % got relief with 100 mg. daily orally.
 Majority got relief with 200 mg. daily orally.
 All responded to 500 mg. daily.

Table 8. Pellagra-preventive Foods.
 (After Sebrell)

Food	Quantity to Prevent Pellagra (gm.)	Food	Quantity to Prevent Pellagra (gm.)
Wheat germ	150	Peanut meal	200
Buttermilk	1200	Liver extract	equiv. 100 gm. liver
Beef, canned, corned	200	Brewer's yeast, dried	30
Beef, fresh lean	200	Evaporated milk	15 cc. per kilo body wt.
Chicken, canned	325	Dry skim milk	105
Liver, pork	24	Fresh skim milk	30 cc. per kilo body wt.
Pork shoulder, lean	200	Dried egg yolk	100
Rabbit	184	Haddock, canned	340
Salmon, canned	168	Beans, kidney, red	360
Collards, canned	482	Beans, soy	360
Kale, canned	534	Green cabbage, canned	482
Peas, green, canned	450	Cow peas	178
Tomato juice, canned	1200	Mustard greens, canned	533
Turnip greens, canned	482	Dried peas	360
Baker's yeast, dried	30	Spinach, canned	482
Baker's yeast, dry and heat-treated	60		
Rice polishings	400		

Elvehjem (1939-40) puts the daily human preventive requirement at 25 mg.

Both pellagrins and normal adults usually respond to initial dosage with nicotinic acid with flushing, burning, and itching sensations, but according to observers these are transitory and accompanied by no harmful effects (Sebrell

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and Butler, 1938) and should not be allowed to interrupt treatment.

Spies, Vilter and Ashe (1937) say of dosage:

“Although the optimal dosage probably varies considerably for different pellagrins, experience with a large series has shown that 500 mg. of nicotinic acid administered daily in 50 mg. doses is safe and effective for the average patient with pellagra. We have observed that only 50 mg. daily may be required for mild pellagra but that in rare instances as much as 1000 mg. daily may be required for very severe pellagra. Administered parenterally, the total daily dose varies from 40 to 80 mg., dissolved in sterile physiologic solution of sodium chloride and injected intravenously in divided doses of from 10 to 15 cc. each. The dose of nicotinic amide and sodium nicotinate is similar to that of nicotinic acid. The oral administration of ten doses of 50 mg. each at hourly intervals is more effective than administration of a single dose of 500 mg. This suggests that the controlling factor is the concentration of compounds of nicotinic acid in the blood and tissues.”

How Does Nicotinic Acid Function?

In Chapter Two we noted that nicotinic acid could act as a hydrogen carrier in an oxidation system. Nicotinic acid amide has been shown to be a component of cozymase or coenzyme I and of coenzyme II. This structure is shown on p. 24. Von Euler (1935) showed that this nucleotide could act as a codehydrogenase, and Warburg (1934) showed that the action was made possible by the presence of the nicotinic amide.

In 1939, Vilter *et al.* utilized the fact that the influenza bacilli require for growth the presence of a substance containing the nicotinic amide or acid in the prosthetic group (coenzyme I or II) to test for presence of this substance in

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human bloods. Their tests showed marked reduction of the factor in pellagrins' blood when contrasted with that of normal individuals, and prompt restoration of the substance to normal content in pellagrin blood after nicotinic acid treatment. It is now fully established that the substance involved is coenzyme I and perhaps II and that both nucleotides contain nicotinic amide (Dorfman *et al.*, 1939). They obtained response of influenza bacilli to both nicotinic acid and coenzyme I. Elvehjem has studied the effect of black-tongue in dogs on both blood coenzyme and tissue storage of the same. He found in dogs that blood changes were slight but tissue storage content significant.

Table 9. Coenzyme I Content in Micrograms per Gram of
Fresh Tissue

Species	Liver	(After Elvehjem)			
		Kidney Cortex	Brain (Gray Matter)	Gastrocn. Muscle	Blood
Human					20-35
Dog	1185	1060		458	51-66
Chick	878	990	306	693	65-105
Rat	1114	1077	353	782	84-106

These observations indicate that nicotinic acid and derivatives, by providing building material for the conduct of normal cell metabolism, may in that way function to keep tissue responses normal and healthy. From that viewpoint we would look at vitamin P-P or nicotinic acid, not as a specific corrective of a special dermatitis but as an agent necessary to keep tissue metabolism normal, just as B₁ is essential to normalizing nerve metabolism in prevention of polyneuritis. Cleckley, Sydenstricker, and Geeslin (1939) have shown nicotinic acid to be valuable in treatment of typical psychotic states.

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Pellagra A Complex

Spies and others have repeatedly emphasized that, although nicotinic acid is specific for correction of the glossitis and dermatitis of pellagra, the ordinary pellagrin represents a resultant of more than one dietary deficiency, *i.e.*, is more than a nicotinic acid deficiency case. Spies (1939) has given the comparison shown in Table 8 showing the relation of the ordinary pellagrin's diet to the nutritive requirements of the human adult:

Table 10.
(After Spies, Vilter and Ashe)

	Pro- tein (gms.)	Calo- ries	Ca ———	P (gms.)	Iron ———	International Units			
						A	B ₁	C	B ₂
Quantity nutrients desirable for adult human:	66	3000	0.68	1.32	0.015	5600	150- 385	150- 375	600- 800
Quantity supplied by average pel- lagrin's diet:	25	1891	0.071	0.396	0.005		38		

These figures (Table 10) support Spies' contention that in treating actual pellagra cases nicotinic acid alone may not be adequate, and that satisfactory treatment involves building up the diet to complete adequacy in all nutrient factors. It also explains why it has been found that B₁, riboflavin and sometimes iron, vitamin C, B₆, or protein correction has been necessary to full recovery.

In line with these findings Spies (1939) has shown a specific role of B₆ in certain conditions encountered in pellagra treatment:

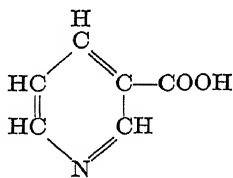
"We described recently the study of a large series of undernour-

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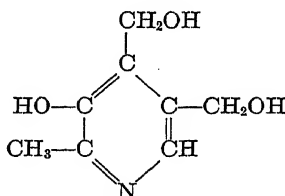
ished persons who had clinical evidence of pellagra and beriberi and certain symptoms which are corrected by the administration of riboflavin. Such persons are greatly benefited by the addition of nicotinic acid, thiamine chloride, and riboflavin to their usual inadequate diets. Some of them regain sufficient strength to return to work, thus enabling them to afford a better diet and thereby be restored to good health. Those whose diets remain unchanged develop symptoms which are not corrected by the addition of these synthetic chemical substances. Such symptoms include extreme nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking.

"Four persons who had been treated successfully for pellagra and beriberi, but who remained on their delcient diets and were now complaining of these symptoms, were selected for treatment. Within four hours after the administration of 50 mg. pure synthetic vitamin B₆ in sterile physiological solution of sodium chloride, all patients experienced dramatic relief of these symptoms and increased strength. Within twenty-four hours these symptoms had disappeared. One of these persons who had been unable to walk more than a few steps walked 2 miles within 24 hours after the injection of 50 mg. of vitamin B₆."

It is interesting to note that B₆, like nicotinic acid, is also a pyridine derivative:



Nicotinic Acid

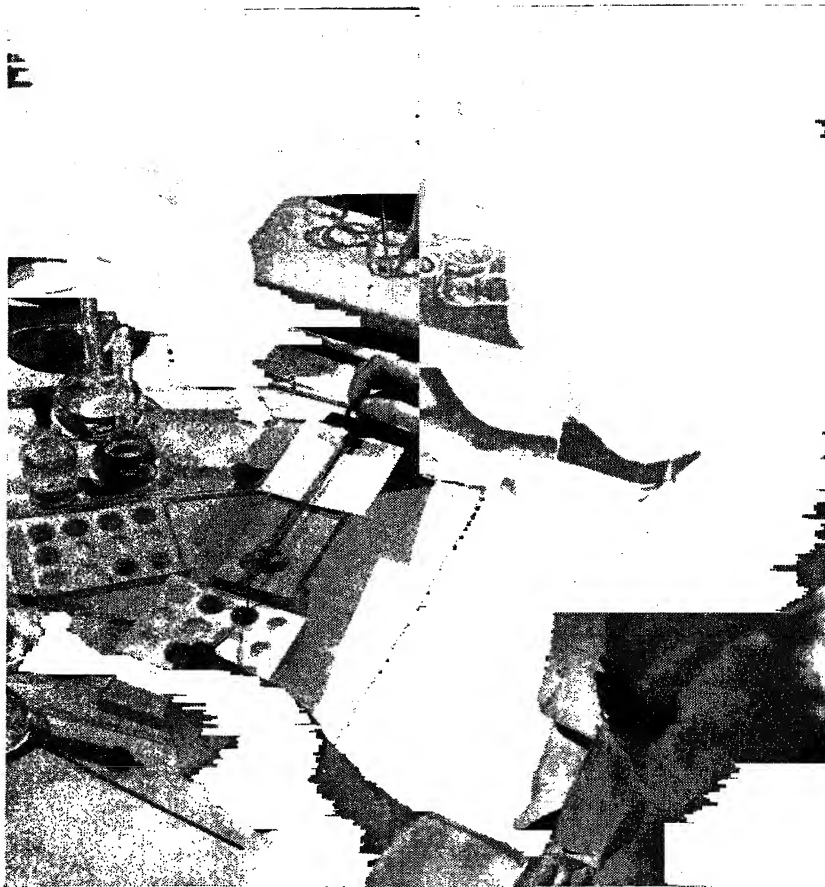


Vitamin B₆

Summary

We have noted above the dosage of nicotinic acid and its role in the correction of the specific lesions of pellagra. Nicotinic acid is stable, non-hygrosopic, and its activity is

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Courtesy Merck & Co., Inc.

Chemical Standardization of a Vitamin by a Microtitration Method

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not destroyed by heat-treatment. It is readily soluble in water, 1 part in 100 parts water at 76° F., soluble in alcohol and readily soluble in a solution of alkali carbonates. It is therefore possible to administer it in different ways in addition to oral treatment.

In nicotinic acid, amide, etc., we have, then, a specific treatment for the lesions of pellagra. Black-tongue in dogs appears to be due to the same causes and is equally satisfactorily relieved by nicotinic acid treatment.

There still remains, however, the prevention as well as the cure of pellagra. The wide extent of this disease in the United States and especially in the southern regions makes it a real menace to the health of a large proportion of the American public. We therefore need specific analyses of the distribution of nicotinic acid in common foodstuffs and a dietary regimen which will supply this substance in adequate amounts for prevention of the disease.

In order to accomplish this, we are in urgent need at the present time of a quick method of assaying the content of nicotinic acid in food and also of measuring nicotinic acid content of biological fluids.

Bibliography

- Cleckley, H. M., Sydenstricker, V. P., and Geeslin, L. E., *J. Amer. Med. Assn.*, 112, 2107 (1939). Nicotinic Acid and Psychotic States.
- Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F., *J. Infect. Dis.*, 65, 163 (1939). Relation of Nicotinic Acid to Growth of Influenza Bacilli.
- Elvehjem, C. A., *Physiol. Reviews*, 20, 249 (1940). The Relation of Nicotinic Acid to Pellagra. Harvey Soc. Lectures, 1939-40. "The Biological Significance of Nicotinic Acid."
- Elvehjem, C. A., Madden, R. J., Strong, F. M., and Wooley, D. W., *J. Biol.*

WHAT ARE THE VITAMINS?

- Chem.*, 123, 137 (1939). See also: *J. Am. Chem. Soc.*, 59, 1767 (1937). The Isolation and Identification of the Anti-Black-tongue Factor.
- von Euler, H., Albers, H., and Schlenck, F., *Z. f. physiol. chem.*, 237, 1 (1935). Concerning the Cozymase.
- Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *Proc. Soc. Exper. B. & M.*, 37, 405 (1937). Treatment of Human Pellagra with Nicotinic Acid.
- France, R., Bates, R. D., Barker, W. H., and Matthews, E., *Bull. Johns Hopkins Hosp.*, 63, 46 (1938). Two Cases of Pellagra Treated with Nicotinic Acid.
- Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 121, 255 (1937). Further Studies of Factor W.
- Funk, C., *J. Physiol.*, 43, 395 (1911). On the Chemical Nature of the Substance Which Cures Polyneuritis in Birds Induced by a Diet of Polished Rice. See also: *J. Physiol.*, 46, 173 (1913).
- Funk, C., and Funk, T. C., *Proc. Soc. Exp. B. & M.*, 119, 35 (1937). Relation of Nicotinic Acid to Growth Stimulation.
- Goldberger, J., and Sebrell, W. H., "Tice's Practice of Medicine," 1932. Treatment of Pellagra.
- Harris, L., Address before Birmingham Univ., Biological Soc., Dec. 9, 1937. See also: *Nature*, 140, 1070 (1937). Preliminary Treatments with Nicotinic Acid on Pellagra.
- Harris, L. J., and Raymond, W. D., *Chem. Ind.*, 58, 652 (1939). Estimation of Nicotinic Acid in Foodstuffs.
- Kringstad, H., and Naess, T., *Z. f. physiol. Chem.*, 260, 108 (1939). Method of Assay for Nicotinic Acid.
- Ruffin, J. M., and Smith, D. T., *Southern Med. J.*, 32, 40 (1939). Treatment of Pellagra with Special Reference to the Use of Nicotinic Acid.
- Schmidt, H. L., and Sydenstricker, V. P., *J. Am. Med. Assn.*, 110, 2065 (1938). Nicotinic Acid in the Prevention of Pellagra.
- Sebrell, W. H., and Butler, R. E., *J. Am. Med. Assn.*, 111, 2286 (1938). A Reaction to Oral Administration of Nicotinic Acid.
- Smith, D. T., and Ruffin, J. M., *Arch. Int. Med.*, 59, 631 (1937). Effect of Sunlight on Clinical Manifestation of Pellagra.
- Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. Amer. Med. Assn.*, 109, 2054 (1937). Pellagra Successfully Treated with Nicotinic Acid.
- Spies, T. D., *Arch. Int. Med.*, 56, 920 (1935). Relationship of Pellagrous Dermatitis to Sunlight.

VITAMIN P-P

- Spies, T. D., Bean, W. B., and Stone, R. E., *J. Amer. Med. Assn.*, 111, 584 (1938). The Treatment of Subclinical and Classic Pellagra.
- Spies, T. D., Gross, E. S., and Sasaki, Y., *Proc. Soc. Exp. B. & M.*, 38, 178 (1938). Effect of Yeast and Nicotinic Acid on Porphyrinuria.
- Spies, T. D., Vilter, R. W., and Ashe, W. F., *J. Amer. Med. Assn.*, 113, 931 (1939). Pellagra, Beri-beri, and Riboflavin Deficiency in Human Beings.
- Sydenstricker, V. P., Schmidt, H. L., Fulton, M. C., New, J. S., and Geeslin, L. E., *Southern Med. J.*, 31, 1155 (1938). Treatment of 45 Cases of Pellagra with Nicotinic Acid.
- Vilter, S. P., Spies, T. D., and Mathews, A. P., *J. Biol. Chem.*, 125, 85 (1938). A Method for the Determination of Nicotinic Acid and Amide.
- Vilter, R. W., Vilter, S. P., and Spies, T. D., *J. Amer. Med. Assn.*, 112, 420 (1939). Relationship between Nicotinic Acid and a Codehydrogenase (Cozymase).
- Walker, W. P., and Wheeler, G. A., *U.S.P.H. Report*, 46, 851 (1931). Influence on Epilepsy of Diet Low in Pellagra-Preventive Factor.

CHAPTER SEVEN

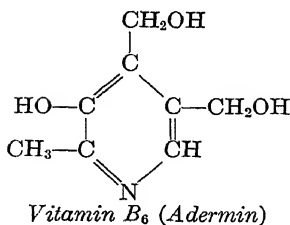
FUNCTIONS OF VITAMIN B₆

CONTINUING the search for the factors present in the vitamin B complex it was discovered that a specific type of rat skin lesion accompanied by a pink or florid dermatitis (acrodynia) resulted from lack of a factor which was first called B₆, then adermin and now pyridoxine. This was first described as filtrate factor I by Lepkovsky, Jukes and Krause (1936). It is apparently identical with the vitamin H of Booher (1937), the H of Hogan and Richardson (1936) and the Y factor of Chick and Copping (1930).

The vitamin was isolated and chemically identified in six laboratories in the same year (Lepkovsky, P. György, Kuhn, Ichiba, Emerson and Keresztesy). Keresztesy and Stevens (1938) in the Merck Laboratory reported the empirical formula of the isolated vitamin as $C_8H_{11}NO_2$. Harris and Folkers (1939) confirmed this by synthesis and established the structure shown in the following figure. This synthetic compound, like the isolated vitamin, cured acrodynia in rats in fourteen days with a dosage of 0.1 mg. daily:

We have already noted that this vitamin, like the anti-pellagic factor, may supply a prosthetic group for cell respiration.

VITAMIN B₆



It will be seen from the structure that in this, as in nicotinic acid, we have the pyridine ring with certain side chains. Crystals of this product are salty tasting, water-soluble and relatively resistant to heat. The product is stable to strong acids, to alkalis, and to nitrous acid. Kuhn found it non-dialyzable from yeast, which may indicate that it exists in the cell in combination with protein as stated in Chapter Two. Its structure indicates that there is probable association with an oxidation enzyme system.

Schneider *et al.* (1939) defined the unit as the amount necessary to cure acrodynia of moderate severity in a rat in three weeks. György (1934) and Wilson and Roy (1938) used a slightly different unit, namely, the amount necessary to cure acrodynia in two weeks. They found the amount to be 0.1 mg. of the pure crystals.

Its distinction from filtrate factor is explained by the following statement of Lepkovsky, Jukes and Krause (1936):

"It has been found that vitamin B and G (flavin) may be readily removed from a solution such as an aqueous extract of rice bran by means of a relatively small amount of fuller's earth. Further treatments with fuller's earth removes, much less readily a third factor, related to the prevention of rat dermatitis. There remains in solution another factor, the 'filtrate factor' which prevents chick dermatitis. For convenience, the factor preventing rat dermatitis will be referred to as Factor I and the filtrate factor, preventing chick dermatitis, as Factor II".

WHAT ARE THE VITAMINS?

The term "B₆" was given to it by György in 1934, who defined it as the part of the B complex curative of a specific dermatitis developed in young rats on a B complex-free diet supplemented only by B₁ and riboflavin. It was first called adermin, but György (1939) suggested that in view of the present established structure of B₆, it should be named "pyridoxine" and that name has now been adopted. The rat dermatitis for which it is specific is characterized by a symmetrical dermatosis affecting first the paws and tips of ears and nose. These areas become red and swollen.

Lepkovsky (1938) claims that the florid dermatitis of the peripheral parts of the bodies of rats on B₆-deficient diets was cured promptly with a daily dose of 10 micrograms, and that 5 micrograms would clear it up, but more slowly. This vitamin also produced gain in weight in animals that had ceased to grow on a B₆-free diet.

Eddy and Dimick (1938) found that when rats were placed on a basal diet completely free of B complex and were given daily supplements of 85.5 micrograms of thiamin, 25 mg. of riboflavin, 50 mg. of nicotinic acid, and 20 mg. of crystalline B₆, the animals showed no appreciable growth and died in the fourth or fifth week. These results indicated that vitamin B₆ like riboflavin, stimulates growth only in the presence of others factors in the B complex not yet isolated.

Birch (1938) showed that the unsaturated fatty acids of maize oil were effective in alleviating the symptoms of vitamin B₆ deficiency. Birch, however, could find no evidence of combination of the vitamin with the lipoids but suggested that there was a functional relation between the vitamin and the unsaturated fatty acids.

Quackenbusch, Platz, and Steenbock (1939) reported that

VITAMIN B₆

rats were protected from acrodynia and continued in good health when maintained on B₆-deficient diet supplemented with unsaturated fatty acids either as natural oils or by giving 10 mg. per day of the ether linoleic acid ester. Salmon (1940) claims that both B₆ and fatty acids are necessary for growth. Dimick and Schreffler (1939) found that rats deprived of B₆ rarely lived more than fifty to sixty days and that these rats showed complete atrophy of the thymus gland and complete absence of fat storage.

The most complete report on distribution at present writing is that of Schneider, Asham, Platz and Steenbock (1939). As stated above, they define their unit as the total amount of source necessary to cure an acrodynia of moderate severity in three weeks. Reference to Table 11 shows that there is a definite relation between fatty material and B₆ distribution, the vegetable oils being exceedingly rich in this factor.

We know little as yet of the value of this factor in human nutrition. We have already referred to Spies' findings (Chapter Six) of its importance to pellagra. Spies, Bean and Ashe (1939) have extended their earlier studies of these syndromes and also the urinary excretion of B₆ after injection into such patients and into normal human individuals. They found that normals excreted 7.9-8.6% of the injected dose, the deficient cases only 0.2%.

Fouts *et al.* (1938) claimed that puppies develop severe microcytic and hypochromic anemia in the absence of B₆ which is cured by the addition to the diet of this missing factor, and György *et al.* (1937) describes a blood system derangement which they call panmyelophthisis in B₆ deficiency in rats.

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Table II. The Anti-acrodynic Potency of Foods.

[After Schneider, Asham, Platz and Steenbock, *Journal of Nutrition*, 17, (1939)].

Food	Units per 100 gm.	Food	Units per 100 gm.
Lettuce	25	Whole wheat bread	400
Spinach	66	Oatmeal	330
Tomato	25	Flaxseed	1000
Potato	40	Rice polish	500
Carrot	25	Wheat germ	1250
Beet	13	Beef tallow	330
Banana	66	Butter fat	200
Orange	16	Lard	2500
Apple	25	Linseed oil (comm.)	2500
Egg yolk	2500	Linseed oil (crude)	2500
Milk whole	40	Peanut oil (ether extract)	5000
Milk skim	14	Peanut oil (benzine extract)	5000
Cheese Cheddar	250	Peanut oil (crude)	2500
Beef muscle (raw, dried)	125	Rice oil (comm.)	2500
Beef muscle (roasted, dried)	125	Rice oil (ether extract)	5000
Haddock (dried)	200	Soy bean oil (ether extract)	1000-7500
Pork liver (dried)	500	Wheat germ oil (comm.)	25000
Alfalfa leaves	600	Wheat germ oil (ether extr.)	15000
Beans, navy	400	Corn oil (comm.)	20000
Peanuts	1660	Dried yeast	400
Soy beans	1250		
Cornmeal	400		

N.B. A unit equals the amount necessary to cure moderately severe acrodynia in 3 weeks.

Bibliography

- Birch, T. W., *J. Biol. Chem.*, 124, 725 (1938). The Relation between Vitamin B₆ and the Unsaturated Fatty Acids. See also: Birch, T. W., and György, P., (1936). A Study of the Chemical Nature of Vitamin B₆.
- Booher, L., *J. Biol. Chem.*, 119, 223 (1937). The Concentration and Properties of Vitamin H.
- Chick, H., and Copping, A. M., *Biochem. J.*, 24, 1764 (1930). Dietary Factors in Addition to the B₁ and B₂: "Y" Factor.
- Dimick, M., and Schreffler, C. B., *J. Nutr.*, 17, 23 (1939). The Factor I Requirement of the Rat.

VITAMIN B₆

- Eddy, W. H., and Dimick, M., *Medical Record*, **148**, 71 (1938). Other Factors of the B Complex than B₁, B₂, B₆, and Nicotinic Acid Necessary for Growth.
- Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *J. Biol. Chem.*, **119**, xxxiv (1937). Effects of Deficiencies of Rat and Chick Anti-dermatitis Factor on Puppies.
- György, P., *Nature*, **133**, 498 (1934). Vitamin B₂ and the Pellagra-like Dermatitis in Rats.
- György, P., *Am. Chem. Soc.*, **60**, 983 (1938). Crystalline Vitamin B₆.
- György, P., and Eckhardt, E., *Nature*, **144**, 512 (1939). Vitamin B₆ and Skin Lesions in Rats.
- György, P., Goldblatt, H., Miller, F., and Fulton, R., *J. Exper. Med.*, **66**, 579 (1937). Panmyelophthisis with Hemorrhagic Manifestations in B₆ Deficiency.
- Harris, S. A., and Folkers, K., *Science*, **89**, 347 (1939); *J. Am. Chem. Soc.*, **61**, 1237, 1242 (1939). Synthesis of Vitamin B₆.
- Hogan, A. G., and Richardson, L. R., Bull. 241, Mo. Agric. Sta. Bull; *Science*, **83**, 17 (1936). Differentiation of the Antidermatitis Factor.
- Ichiba, A., and Michi, K., *Sci. papers Inst. Phys. & Chem. Res., Tokyo*, **34**, 623 (1938). Crystalline B₆.
- Keresztesy, J. C., and Stevens, J. R., *Proc. Soc. Exp. B. & M.*, **38**, 64 (1938). Crystalline B₆.
- Kuhn, R., and Wendt, G., *Ber. Deutsch chem. Ges.*, **71**, 780 (1938). Concerning the Antidermatitis Factor in Liver.
- Lepkovsky, S., *J. Biol. Chem.*, **124**, 125 (1938). The Isolation of Factor I in Crystalline Form.
- Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, **115**, 557 (1936). The Multiple Nature of the Third Factor of Vitamin B Complex.
- Quackenbusch, F. W., Platz, B. R., and Steenbock, H., *J. Nutr.*, **17**, 115 (1939). Rat Acrodynia and the Essential Fatty Acids.
- Salmon, W. D., and Goodman, J. G., *J. Nutr.*, **13**, 477 (1937). Alleviation of Vitamin B Deficiency in the Rat by Certain Natural Fats and Synthetic Esters.
- Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., *J. Nutr.*, **18**, 99 (1939). The Anti-Acrodynic Properties of Certain Foods.
- Spies, T. D., Bean, W. B., and Ashe, W. F., *J. Amer. Med. Assn.*, June 10, 1939.
- Wilson, H. E. C., and Roy, G. K., *Indian J. Med. Res.*, **25**, 879 (1938). The Flavin and Vitamin B₆ Content of Certain Indian Foodstuffs.

CHAPTER EIGHT

FUNCTIONS OF OTHER MEMBERS OF THE B COMPLEX

IN THE preceding chapters we have dwelt on the chemical identification and synthesis of four members of what in 1916, McCollum and Kennedy called water-soluble B, namely, B₁ or thiamine, B₂ or riboflavin, B₆ or pyridoxine, and P-P or nicotinic acid.

If water solubility is a characteristic of this complex we must also record the postulation of several more members of the complex, members already listed in Table 1, p. 5. To date, there has been little evidence adduced to show the human need for most of these other vitamins, but it must be borne in mind that lack of evidence is not proof of lack of value in human nutrition. Until Spies showed the relation of B₆ to lesions in human pellagrins our only evidence of its need was the prevention of rat dermatitis.

It would seem worthwhile, therefore, at least to describe the functions of these B-complex factors in the test animals used. Their chemical identification and availability may, when attained, provide means for study of their value to man and perhaps reveal importance for the human diet.

The following, then, is a review of what these factors

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appear to effect in diets. The only member fully established as to chemical nature in addition to vitamins B₁, B₂, B₆, and nicotinic acid is pantothenic acid or filtrate factor.

Vitamin B₃

Williams and Waterman (1928) found that when pigeons were fed on polished rice and water to produce typical avian polyneuritis the addition of highly concentrated solutions of B₁ corrected the neuritic symptoms but failed to restore the birds to normal weight. Supplementing the diet with heat-treated yeast failed to bring about weight recovery, but air-dried yeast was quite effective. These tests led them to infer the existence of a heat-labile factor in yeast other than B₁ or B₂, and to postulate in 1928 the "tripartite" nature of the B complex. The existence of such a heat-labile factor and its requirement by chicks as well as pigeons was confirmed by Eddy, Gurin and Keresztesy (1930) and by O'Brien (1934), though the latter found the heat-lability to vary considerably and suggested that such variations might be occasioned by the state of combination of the vitamin and its natural source.

In 1936, Almquist and Stokstad reported that a factor (first noted by Dam), whose absence from chick diet resulted in gizzard erosion, could be cured by the saponifiable fraction of hexane extract of alfalfa. The nature of this gizzard erosion factor was investigated by Bird, and others (1936). Of it they state:

"At an early stage in this work, the heat lability of the factor as it occurs in grains suggested the possibility of its identity with B₃. The later discovery of the greater stability of the factor as it occurs in

WHAT ARE THE VITAMINS?

lung seemed to argue against this possibility, but this greater stability may have been due merely to a greater concentration originally present. . . . A pigeon experiment gave inconclusive results although pigeons fed on polished rice supposedly deficient in B₃ did show slight gizzard lesions. . . . Disagreements in the literature as to the properties of vitamin B₃ make it difficult to establish definitely the identity or non-identity of the two factors."

Carter and O'Brien (1940) have recently suggested that this factor may be identical with pantothenic acid, and since that product is now available in pure crystalline form it should be possible to determine this point.

Vitamin B₄

Reader (1929-30) reported a heat-labile, water-soluble factor different from B₁, B₂ or B₃ which prevented a sort of paralysis in rats characterized by hunched back, lack of coördination, and swollen paws. Kline and associates (1935) at Wisconsin confirmed the existence of this factor; and it has been concentrated but not isolated from yeast extracts and defatted liver. Human need for the factor has not been demonstrated but it is apparently required by chicks (Keenan *et al.*, 1935) as well as by rats.

Vitamin B₅

That pigeons require for prevention of weight loss a heat-stable factor other than B₂, led Carter and Kinnersley and Peters (1930) to postulate the existence of a B₅ factor. B₅ prevented only weight maintenance of the pigeon; B₃ was necessary for weight increase. These are the only reports in the literature on this factor with the exception of a recent

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paper by Carter and O'Brien who suggest that it may be identical with B₆ or pyridoxine.

Filtrate Factor (Pantothenic Acid)

Referring back to p. 109, we find Lepkovsky, Jukes and Krause's (1936) original definition of Filtrate Factors I and II. At that time it had been established that when, from an extract of such a substance as liver, one removed vitamins B₁ and B₂ (riboflavin) the resulting filtrate still showed effect on certain dermatoses of rats, chicks and dogs.

The isolation of nicotinic acid cleared up the relation to blacktongue in dogs and human pellagra, but the resulting filtrate still proved protective against rat and chick dermatitis. Lepkovsky *et al.* in 1936 suggested (see quotation, p. 109) that the filtrate still contained at least two distinct vitamins, one curative of rat dermatitis (Factor I) and one of chick dermatitis (Factor II). With the isolation of B₆, or pyridoxine, it was felt for a time at least that the rat factor had been found; filtrate factor thus came to mean chick dermatitis corrective factor only, in the Lepkovsky, Jukes, Krause nomenclature.

Nelson (1938) defines filtrate factor as follows:

"The name 'Filtrate Factor' was proposed provisionally to refer to a member of the vitamin B complex which had been demonstrated in earlier investigations at Cornell and Wisconsin to prevent a dermatitis in chicks. The term was chosen to refer to the method of preparation of concentrates containing the factor used by Elvehjem and Koehn (1937) but has also been used in referring to the fraction containing the vitamin. Subsequent studies, even as late as 1938 report that filtrate factor preparations are effective in curing blacktongue in dogs or human pellagra. The studies that have been made

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now make it clear that if the term 'Filtrate Factor' is to be retained it should be used only as originally defined, viz. *a factor which prevents a nutritional dermatitis (or perhaps preferably a dermatosis) in chicks*".

These statements are necessary to make clear what is now generally understood by the term "Filtrate Factor" among vitamin investigators, but fortunately all confusion has now been eliminated, together with the term "Filtrate Factor" itself, by proof of its identity with the pantothenic acid of R. J. Williams (1939), an early investigator in the field of yeast growth stimulants. At one time he was led to the belief that Wildier's (1901) "bios" (the wort constituent essential to yeast growth, according to Wildier) was identical with water-soluble vitamin B. Pursuing his studies of the water-soluble B complex, he reported in 1939 a compound whose calcium salt appeared to have the formula $(C_8H_{14}N_2O_5)_2 Ca$. He found this substance so universally distributed in plant and animal tissues and apparently essential for growth of all living cells that he coined for it the name "pantothenic acid".

Science Service reports R. J. Williams as saying:

"Since its discovery pantothenic acid has been found to be not only present in widely different tissues and organisms but to function as a patent physiological substance stimulating the growth of yeasts, molds, lactic acid bacteria, diphtheria bacillus, protozoa, young alfalfa seedlings and liver worts, and to stimulate the respiration of various tissues.

"The present discovery of Jukes and of Wooley, Waisman, and Elvehjem is the first one linking it up definitely as a 'growth-promoting' substance for higher animals, though it has been recognized as a constituent of all types of animal tissue and to be stored in the livers of all animals.

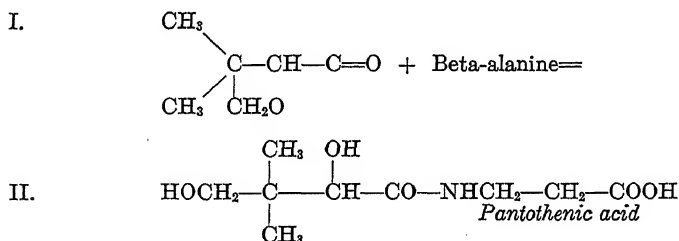
"There is evidence that the same substance is required by pigs and dogs and the inference is not a wild one that it is necessary for the

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nutrition of all the higher forms of animal life and that it makes up an essential part of every living cell."

Jukes (1937) reported a method for assay of filtrate factor and its distribution in certain food products. He defined a filtrate factor unit as follows: One unit of the filtrate factor is one-tenth the amount which will just provide for maximal growth when fed daily to a chick 3 weeks old in conjunction with a heated diet under conditions described by Jukes. Jukes (1939) and Wooley (1939) produced evidence that pantothenic acid is identical with the chick dermatitis corrective factor (Filtrate Factor).

Recently (1940) a group of chemists at the Merck Laboratories coöperating with Dr. Williams and his group have successfully determined the structure of pantothenic acid and have confirmed it by synthesis. The following structure shows how it is formed as an alanine combination and its formula:



The following tables show its distribution in certain foods as determined by Jukes:

Further details of the methods of assay are given by Jukes (1939).

For details of formula determination and synthesis the reader is referred to the papers by Williams, Mitchell, Wein-

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Table 12. Filtrate Factor Values of Some Feedingstuffs and Human Foods.

(After Jukes)

Some of the foods were dried before being fed, but all calculations are on the basis of undried weight.

Material	Amount Fed in Ration (%)	Filtrate Factor Value (unit per gm.)	Mean Filtrate Factor Value (unit per gm.)
Rice bran extract, Type II (by volume)*	3	20	20
Baker's yeast 3 (unirradiated)†	3 4	15 21	18
Baker's yeast 3 (after irradiation)†	3 4	18 19	18
Peanut meal‡	25	3.2	
	25	3.8	3.5
Soybean meal 1	20	1.2	
	25	1.6	1.4
Soybean meal 2‡	20	0.7	
	25	0.6	0.6
Cottonseed meal‡	20	1.3	
	25	0.8	1.0
Sesame meal‡	20	0.4	
	25	0.4	0.4
Linseed meal‡	10	<0.2	<0.2
	25		Injurious
Coconut meal‡	15	<0.2	
	15	<0.2	<0.2
Babassu meal‡	15	<0.2	<0.2
Hexane-extracted wheat germ	30	0.7	0.7
Ground white milo	58	0.6	
	80	0.7	0.6
Rice bran 2	30	1.9	1.9
Alfalfa leaf meal	10	1.3	1.3
Beef round, dried at 70° in vacuum oven	25 50	0.8 0.8	0.7

* Obtainable from Vitab Products, Inc., of San Francisco, who kindly supplied it.

† Supplied by Standard Brands, Inc., of New York, by the kindness of Dr. C. A. Smith.

‡ Supplied by the Poultry Producers of Central California, through the courtesy of Dr. George Kernohan.

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Table 12 (*Continued*)

Material	Amount Fed in Ration (%)	Filtrate Factor Value (unit per gm.)	Mean Filtrate Factor Value (unit per gm.)
Onions, dried at 50°	38	<0.2	
	58	<0.2	<0.2
Carrots, dried at 50°	58	0.2	
	116	<0.2	<0.2
Canned green peas, dried at 50°	54	<0.2	
	54	<0.2	<0.2
Fresh green peas, dried at 50°	110	0.4	0.4
Dried green peas (split peas)	25	1.4	
	40	1.6	1.5
Cow peas	62	1.2	
	62	1.4	1.3
Navy beans, raw	62	Injurious	
Navy beans, heat-treated	62	<0.2	<0.2
Rolled oats	80	0.8	
	50	0.8	0.8
Egg white, boiled 30 min.	12	<0.2	
	50	0.2	
	80	<0.2	<0.2
Egg yolk, boiled 30 min.	14	3.7	
	10	4.2	4.0
Canned salmon, dried at 70° in vacuum oven	40	0.7	
	40	0.5	0.6

stock and Snell (1940), and Stiller, Harris, Finkelstein, Keresztesy, and Folkers (1940) (see bibliography).

At the present writing only one paper dealing with the value of pantothenic acid in human nutrition is available. (Spies, Stanbery, Williams, Jukes, Babcock, 1940.) These investigators report the following findings:

- (1) Administration of varying amounts of calcium and sodium pantothenate to 15 persons produced no sig-

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Table 13. Comparison of Filtrate Factor Content of Certain Foods with Pellagra-Preventive Values of Similar Foods as Determined by Goldberger and Co-workers.

The weights are on an undried basis unless otherwise stated.

Food	Amount Containing 1 Unit of Filtrate Factor (= 1 + Filtrate Factor Value) (gm.)	Human Pellagra- Preventive Value	Daily Amount Fed in Human Pellagra Test (gm.)
Dried egg yolk	0.13	Fair (black-tongue)	
Peanut meal	0.3	Good	200
Dried green peas (split peas)	0.6	Fair	360
Cow peas	0.8	Fair	178
Rolled oats	1.2	None (black-tongue)	
Kale	1.2	Good	534
Wheat germ (fat-extracted)	1.4	Good	150
Fresh beef round	1.5	Good	200
Whole corn-meal	1.7	None	270
Canned Alaska salmon	1.7	Good	168
Carrots	5.0	Slight	450
Mature onions	5.0	None	525
Canned green peas	5.0	Good	450

nificant changes in blood pressure, pulse, temperature, or respiration in doses up to 100 milligrams.

- (2) Stanbery, Snell and Spies have worked out a method for the assay of pantothenic acid in blood and urine and using this method before and after injections of pantothenic acid, they report that both blood and urine content rise rather quickly after injection (within 3 hrs.) but both blood and urine return to original levels within 24 hours after the injections.
- (3) They found the blood concentration of pantothenic acid

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in pellagrins, beriberi cases, and riboflavin deficient patients 23 to 50 per cent lower than that of 18 normal persons used for comparison.

- (4) They report an interesting relation between pantothenic acid and riboflavin. Using a microtechnique developed by Snell, Strong and Peterson (1939) for determination of riboflavin in blood they report that the injection of pantothenic acid not only increased blood concentration of that factor but simultaneously a 20 to 30% rise in blood riboflavin level. Injections of 20 mg. Calcium pantothenate to riboflavin patients showing cheilosis or ocular B₂ deficiency symptoms also raised the blood riboflavin levels in these cases which returned to the former state when the injections were discontinued. There was also a correlated reaction following riboflavin injection. They found that with injection of 200 micrograms of riboflavin per kilo of body weight the blood flavin content increased 80% and its pantothenic acid content 45%, both returning to the former level the day following the riboflavin injection.
- (5) They conclude that pantothenic acid appears essential to human nutrition and is probably intimately associated with riboflavin in this behavior.

Since pantothenic acid in pure form is now available, progress should be rapid in determining its behavior and function.

Vitamin B₇ or Vitamin I

Centanni (1935) claims to have isolated from alcohol extract of rice polishings a substance which was without effect

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in the prevention of beriberi or polyneuritis but which prevented digestive disturbances in birds. This factor may be identical with that described by Carter (1930) and by Rose-dale (1927). Little is known of it today and there are no data on its value to humans.

Vitamin H (Biotin)

As noted on p. 108 this letter was used by Booher (1933) to describe what is now known as B₆. The letter has also been used by McCay, Bing and Dilley (1928) to designate a factor necessary to the life of the trout. Stepp *et al.* (1937) used the letter to designate a factor set free from liver by digestion with proteolytic enzymes.

Parsons and associates (1934, 1937) reported a type of dermatitis in rats produced by eating uncooked egg white and curable by injection of a material obtained by digesting a liver extract with papain, extracting with water, and re-extracting the dried extract with methanol. This product is probably identical with Stepp's product; P. György (1937) concentrated the factor further and obtained preparations effective in parenteral doses of 3 to 5 mg. György calls this factor vitamin H.

In 1936 Kogl and Tonnies in their study of "bios", or yeast growth stimulation factors, reported the isolation from egg yolk of a crystalline product for which they suggested the name "biotin".

In 1933 Allison, Hoover and Burk reported a factor essential for the respiration of certain lower organisms to which they gave the name "coenzyme". They found it essential to the growth of a legume organism, *Rhizobia*.

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These three discoveries appear now to be dealing with the same substance. György, Melville, Burk and du Vigneaud (1940) report that in chemical properties and physiological action vitamin H, biotin, and coenzyme R are closely similar and probably identical. Since biotin has been obtained in crystalline form by Kogl and Tonnies, though in limited amount, it should be possible to determine definitely whether vitamin H is biotin; if so, vitamin H joins the group successfully isolated and identified.

The substance is dialyzable, heat-stable, and water- and alcohol-soluble, but is not soluble in ether or chloroform. It is readily adsorbed on charcoal but not precipitated by lead acetate. It is inactivated by nitrous acid and by acetylation. It may exist in combination with protein or other colloid, accounting for its release by proteolytic enzymes.

Vitamin J

Von Euler (1935) reported the extraction of a factor from the juice of fruits that was not antiscorbutic, but protected guinea pigs from pneumonia. He called it vitamin J. Its value in treating pneumonia in man has not been demonstrated.

Anti-Gray Hair Factor

In the study of filtrate factor Morgan, Cook and Davison (1938) and Lunde and Kringstad noted that rats on filtrate factor deficiency diets showed marked graying of dark hair. Lunde and Kringstad (1939) confirmed this observation and claimed that some factor (not B₆, Filtrate factor, riboflavin, B₁ or nicotinic amide) deficiency caused the black hair of

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piebald rats to become gray, and the white hair of albino rats to become dirty brown. They found that the product exists in yeast and is less heat-stable than riboflavin.

Mohammad, the Emersons and Evans (1939) reported separation of the "gray hair factor" from the filtrate factor or pantothenic acid. It goes with the ether extractable component of filtrate factor according to their findings. But, more recently György and Poling (1940) claim to have successfully cured graying of hair in rats by a dosage of 75-100 mg. of crystalline pantothenic acid. Cure was complete in 5-7 weeks.

Other Water-Soluble Vitamins Postulated

Factors L₁ and L₂. Nakahara and associates (1938) claim that substances which are necessary to milk formation may be concentrated from beef liver (L₁) and from bakers' yeast (L₂). They consider that they function in the maturation of the lactation tissues.

Factor M. Day (1938) and Langston (1938) report that nicotinic acid is of no value in correcting pellagra symptoms in the Rhesus monkey. Combinations of thiamine, riboflavin and nicotinic acid would not correct the oral lesions. Dried brewers' yeast and liver extract did clear up the symptoms. They have therefore postulated a factor which they designate as "M".

Factor U. Stokstad and Manning (1938) have suggested the name factor "U" for a vitamin apparently essential for chick growth. This factor they found soluble in 50% alcohol, insoluble in ether, acetone, and isopropyl alcohol. It is adsorbable on fuller's earth and charcoal and is destroyed in alfalfa

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by 5 hours' heating at 120° C., but is not destroyed in yeast by heating or refluxing for 30 minutes at pH 1.7 to 11. Its significance in human nutrition is unknown.

Factor W. In addition to B₁, B₂, B₆ and Filtrate Factor, Elvehjem, Koehn and Oleson (1936) have suggested another rat-growth promoting factor to which they gave the letter designation "W". Frost (1937) has suggested its possible relation to the pyridine nucleotides. Elvehjem also describes a rat condition which he calls the "spectacled eye" state. He suggests that it is due to a specific vitamin.

Grass Juice Factor. Koehn, Elvehjem and Hart (1936) postulated that grass juice contains a nutrient factor of a water-soluble nature and grass extracts in concentrated form have been shown to be rich in various vitamin factors. Whether the factor described by Koehn *et al.* is unique and different from all other members of the B complex has not been established.

Borsook and associates (1938) reported a study of the effect of the B complex on 227 cases of functional gastrointestinal malfunction. The B complex was of distinct value in this treatment and the observers make the following statement:

"There are indications from the experimental work on animals and our observations on humans that in most cases the whole B complex is superior therapeutically to any single fraction. There is also the obvious and important economic reason for preferring the whole B complex as it is formed in foods to any highly purified single component."

It is obvious from this review of possible and demonstrated members of the B complex that much remains to be done to clarify the present situation. Only progressive isolation

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Courtesy Merck & Co., Inc.

Catalytic Synthesis in Vapor Phase

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and chemical identification of individual factors can explain what observed effects are due to specific deficiencies and what are due to variation in proportions (synergistic effects) of the various factors.

Bibliography

- Allison, F. E., Hoover, S. R., and Burk, D., *Science*, **78**, 217 (1933). Co-enzyme "R."
- Almquist, H. J., and Stokstad, E. L. R., *Nature*, **137**, 581 (1936). A Nutritional Deficiency Causing Gizzard Erosion in Chicks.
- Barnes, R. E., O'Brien, J. R., and Reader, V., *Biochem. J.*, **26**, 2035 (1932). Vitamin B₁.
- Boas, M. A., *Biochem. J.*, **21**, 712 (1927). The Effect of Desiccation on the Nutritive Properties of Egg White.
- Borsook, H., Dougherty, P., Gould, A. A., and Kremers, E. D., *Am. J. Dig. Dis. & Nutr.*, **5**, 246 (1938). The Vitamin B Complex and Functional Chronic Gastro-intestinal Malfunction.
- Carter, C. W., *Biochem. J.*, **24**, 1811 (1930). Heart Block in Pigeons, Curative Factor Similar to Centanni's B₇.
- Carter, C. W., and O'Brien, J. R., *Biochem. J.*, **30**, 43 (1936). Maintenance Nutrition in the Pigeon; Vitamin B₃ Concentrates. Proc. 7th World's Poultry Congress, p. 126, 1939. Identity of B₃ and Pantothenic Acid.
- Carter, C. W., Kinnorsley, H. W., and Peters, R. A., *Biochem. J.*, **24**, 1832, 1844 (1930). Maintenance Nutrition in the Adult Pigeon B₅.
- Centanni, E., *Biochim. e terap. sper.*, **22**, 137 (1935). The Enteral Vitamin I or B₇, Regulating Digestive Function in Pigeons.
- Day, P. L., Langston, W. C., and Darby, W. J., *Proc. Soc. Exp. B. and M.*, **38**, 860 (1938). Failure of Nicotinic Acid to Prevent Nutritional Cytopenia in Monkeys. Postulation of Vitamin M.
- Eddy, W. H., Gurin, S., and Keresztesy, J. C., *J. Biol. Chem.*, **87**, 729 (1930). The Williams-Waterman B₃ factor.
- Elvehjem, C. A., Koehn, C. J., Jr., and Oleson, J. J., *J. Biol. Chem.*, **115**, 707 (1936). A New Essential Dietary Factor (W).
- von Euler, H., Soder, H., and Malmberg, M., *Ztschr. f. Hyg. u. Infektionskr.*,

WHAT ARE THE VITAMINS?

- 116, 672 (1935). The Action of the Nutrient Factor "J" on the Development of Pneumonia in Guinea Pigs.
- Fixsen, M., and Boas, M. A., *Biochem. J.*, 25, 596 (1931). Further Studies of Effect of Desiccation on the Nutritive Value of Egg White.
- Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 121, 255 (1937). Further Studies on Factor W.
- György, P., *J. Biol. Chem.*, 131, 733 (1939). Properties of Vitamin H.
- György, P., Melville, D. B., Burk, D., and duVigneaud, V., *Science*, 91, 243 (1940). Poss. Identity of Biotin, Coenzyme R and Vitamin H.
- György, P., and Poling, C. E., *Science*, 92, 202 (1940). Pantothenic Acid and Achromotrichia in Rats.
- Hitchings, G. H., and Subarrow, Y., *J. Nutr.*, 18, 268 (1939). The Rat Growth Factor of the Filtrate Fraction of Liver Extract.
- Jukes, T. H., *J. Biol. Chem.*, 117, 11 (1937). Method of Assay of Filtrate Factor. *J. Am. Chem. Soc.*, 61, 975 (1939). Identity of Filtrate Factor and Pantothenic Acid.
- Jukes, T. H., and Babcock, S. H., *J. Amer. Med. Assn.*, 115, 523 (1940). Pantothenic Acid in Human Nutrition.
- Jukes, T. H., and Lepkovsky, S., *J. Biol. Chem.*, 114, 109 (1936). The Effect of Some Reagents on Filtrate Factor.
- Keenan, J. A., Kline, O. L., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *J. Biol. Chem.*, 103, 671 (1935). New Nutritional Factors Required by the Chick.
- Kline, O. L., Elvehjem, C. A., and Hart, E. B., *Biochem. J.*, 30, 780 (1936). Further Evidence of the Existence of B₄.
- Koehn, C. J., and Elvehjem, C. A., *J. Biol. Chem.*, 118, 693 (1937). Further Studies of the Concentration of the Antipellagric Factor.
- Kogl, F., Tonnies, B., *Z. physiol. Chem.*, 242, 42 (1936). Discovery of Biotin.
- Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 115, 557 (1936). The Multiple Nature of the Third Factor of the Vitamin B Complex.
- Lunde, G., and Kringstad, H., *Z. physiol. Chem.*, 257, 201 (1939). Concerning Change of Pelt Color Due to Absence of a B-complex Factor.
- McCay, C. M., Bing, F. C., and Dilley, W. E., *Science*, 67, 249 (1928). Factor H in the Nutrition of Trout.
- Mohammad, A., Emersons, O. H., and G. A., and Evans, H. M., *Science*, 90, 377 (1939). Multiple Nature of the Rat Filtrate Factor.

B COMPLEX

- Morgan, A. F., Cook, B. B., and Davison, H. G., *J. Nutr.*, 15, 27 (1938). Vitamin B₂ Deficiency as Affected by Carbohydrate; Graying of Hair Effect.
- Morgan, A. F., and Simms, H. D., *J. Nutr.*, 19, 233 (1940). Greying of Fur and Other Disturbances in Several Species Due to a Vitamin Deficiency.
- Nakahara, W., and Inukai, F., *Sci. Papers Inst. Phys. and Chem. Res.*, 22, 301 (1933). First Publication on the Existence of Vitamins L₁ and L₂.
- Nakahara, W., Inukai, F., Ugami, S., *Proc. Imp. Acad. Japan*, 14, 9 (1938). Factor L; Requirements for Lactation in Rats.
- Nelson, E. M., *J. Amer. Med. Assn.*, 110, 727 (1938). Components of the Vitamin B Complex.
- O'Brien, J. R., *Biochem. J.*, 28, 926 (1934). Vitamin B₈.
- O'Brien, J. R., *Chem. and Ind.*, 53, 452 (1934). Methods of Assay of Vitamin B₄.
- Oleson, J. J., Wooley, D. W., and Elvehjem, C. A., *Proc. Soc. Exp. B. & M.*, 42, 151 (1939). Is Pantothenic Acid Essential to the Growth of Rats?
- Parsons, H. T., and Lease, J. G., *J. Nutr.*, 8, 57 (1934). Variations in the Potency of Certain Foodstuffs in the Cure of Dermatitis Induced in Rats by Dietary Egg White.
- Parsons, H. T., Lease, J. G., and Johnson, D., *J. Biol. Chem.*, 31, 77 (1937). The Storage in the Body of the Factor Protective Against Injury Due to Egg White.
- Reader, V., *Biochem. J.*, 23, 689 (1929). A Second Thermolabile Water-soluble Accessory Factor. *Biochem. J.*, 24, 1827 (1930). The Assay of Vitamin B₁. *Biochem. J.*, 24, 77 (1930). Further Evidence of a Third Accessory Factor.
- Rosedale, J. R., *Biochem. J.*, 21, 1266 (1927). Preliminary Note on Possible Factor (B₇ ?).
- Snell, E. E., Strong, F. M., and Peterson, W. H., *Ind. Eng. Chem. (Anal. Ed.)*, 11, 346 (1939). Assay for Pantothenic Acid.
- Spies, T. D., Stanbery, S. R., Williams, R. R., Jukes, T. H., and Babcock, S. H., *J. Amer. Med. Assn.*, 115, 523 (1940). Pantothenic Acid in Human Nutrition.
- Stanbery, S. R., Snell, E. E., and Spies, T. D., Note in *J. Biol. Chem.* (1940). Method of Assay of Riboflavin in Blood.
- Stiller, E. T., Harris, S. A., Finkelstein, J., Keresztesy, J. C., and Folkers, K.,

WHAT ARE THE VITAMINS?

- J. Am. Chem. Soc.*, **62**, 1785 (1940). Pantothenic Acid VIII.; the Total Synthesis.
- Stokstad, E. L. R., and Manning, P. D. V., *Science*, **88**, 35 (1938). A New Dietary Water-soluble Factor Required by Chicks; Vitamin U.
- du Vigneaud, V., Melville, D. B., György, P., and Rose, C., *Science*, **92**, 62 (1940). On the Identity of Vitamin H and Biotin.
- Wildier, E., *La Cellule*, **18**, 313 (1901). Concerning "Bios."
- Williams, R. J., *et al.*, *J. Am. Chem. Soc.*, **55**, 2912 (1933). Pantothenic Acid.
- Williams, R. J., Eakin, R. E., and Snell, E. E., *J. Am. Chem. Soc.*, **62**, 1204 (1940). The Relationship of Inositol, Thiamin, Biotin, Pantothenic Acid, and Vitamin B₆ to the Growth of Rats.
- Williams, R. J., Mitchell, H. K., Weinstock, H. H., Jr., and Snell, E. E., *J. Am. Chem. Soc.*, **62**, 1784 (1940). Pantothenic Acid VII. Partial and Total Synthesis Studies.
- Williams, R. R., and Waterman, R. E., *J. Biol. Chem.*, **78**, 311 (1928). The Tripartite Nature of Vitamin B.
- Wooley, D. W., Waisman, H. H., Mickelson, O., and Elvehjem, C. A., *J. Biol. Chem.*, **125**, 715 (1938). Some Observations on the Chick Antidermatitis Factor.

CHAPTER NINE

THE FUNCTIONS OF VITAMIN C

THE elucidation of the chemical nature of the anti-scorbutic vitamin is a product of studies in many laboratories. In 1932, King and Waugh obtained from lemon juice an actively scorbutic substance apparently identical chemically with the "hexuronic acid" recovered from adrenal cortex, oranges and cabbage by Szent-Gyorgyi (1928). The identity of hexuronic acid as the antiscorbutic vitamin itself was announced by Svirbely and Szent-Gyorgyi and by King and co-workers in 1932-33. Its structure was established by Haworth, Hirst and collaborators (1933) and in the same year Reichstein, Grussner and Oppenhauer (1933) synthesized it.

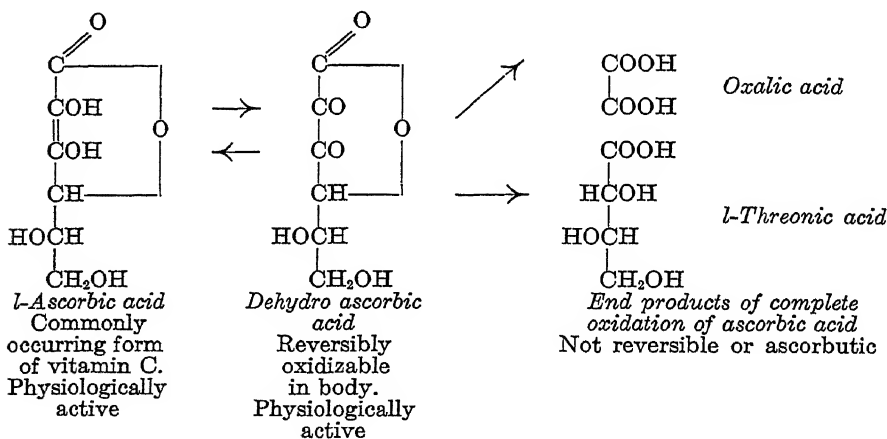
Szent-Gyorgyi's isolation of vitamin C was a consequence of his study of oxidation systems. In his lectures (1939) he gives the following account:

"The more I learned about this new substance, the more interesting it seemed to be. Eventually, I crystallized it, that is to say, peeled it out in a pure condition which made analysis possible. It was an acid and it seemed to be related to an unknown sugar which I called 'Ignose', the substance itself being called 'Ignosic Acid'. But the editor of the journal to whom I sent my paper did not like jokes and rejected the name. 'Godnose' being no more successful, we

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agreed that the child's name should be "hexuronic acid". Later, with advancing knowledge of its structure it had to be rebaptized in haste and it is now called ascorbic acid (sometimes, cevitamic acid) because it is identical with vitamin C and prevents scurvy. In this way I became a father without wishing it, the father of a vitamin. Such accidents seem to happen even in science."

The outstanding characteristic of ascorbic acid is that the oxidation reaction proceeds in two steps, the first step being reversible, the second irreversible. The structure of *l*-ascorbic acid and these oxidative changes are shown below:



It was early shown that the most characteristic feature of vitamin C was the rather rapid destruction of physiological activity by oxidation, especially when the substance was heated in an alkaline or neutral solution. We know now that this is due to the chemical changes shown above. In natural sources the active vitamin occurs mainly in the fully reduced form (*l*-ascorbic acid). If oxidation is not too severe the *l*-ascorbic acid may simply lose two hydrogens and change to the dehydro-ascorbic acid form. This form

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may be reconverted to *l*-ascorbic acid by reducing agents such as hydrogen sulfide, a change which can also be accomplished in the body; hence ascorbic acid eaten in either the *l*- or dehydro- form is available for human use as an antiscorbutic. If, however, the oxidation proceeds further than the dehydro- stage, no reversion to *l*-ascorbic acid is possible and physiologic activity is lost. In assay of vitamin C sources, then, it is necessary to determine content of both *l*-ascorbic acid and dehydro-ascorbic acid to estimate properly their scurvy-preventive potency.

l-Ascorbic and dehydro-ascorbic acids are not, however, the only compounds with antiscorbutic properties though they appear to be the principal forms of the vitamin in foods and biological materials. The following forms have been synthesized and tested for antiscorbutic value with the results noted:

l-rhamno-ascorbic acid; $1/5$ th the potency of *l*-ascorbic acid.

l-arabo-ascorbic acid; $1/20$ th the potency of *l*-ascorbic acid.

d-ascorbic acid; no potency.

d-gluco ascorbic acid; no potency.

d-galacto ascorbic acid; no potency.

It would appear from these products and their behavior that one essential to antiscorbutic activity of these sugar acids is associated with the position of the oxygen ring; the *d*-forms being inactive, the *l*-forms active. This ring position is not the only factor concerned with physiological activity, however, for while the *l*-rhamno and *l*-arabo forms have the ring in the proper position, they exhibit only $1/5$ to $1/20$ the potency of *l*-ascorbic acid itself.

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The Avitaminosis Theory of Scurvy

In 1757, James Lind published his classic "Treatise on Scurvy", the first clear account of the disease. Lind established the efficacy of lemon juice for its prevention and cure. In 1804, Sir Gilbert Blaine secured regulations enforcing supply of lemon juice to the sailors of the British Navy and in 1865, similar regulations were adopted for the mercantile marine. Funk, in his first review of possible avitaminosis, suggested that scurvy might be a vitamin deficiency disease, but Holst and Frohlich (1907) initiated modern research for this vitamin by showing that scurvy could be experimentally produced in guinea pigs.

The characteristic of the vitamin that proved the best clue to its nature was its instability. This was shown by the work of Zilva (1935) in England, of Vedder (1921) and of King (1931) in America and of Bezssonoff (1929-31) in France. In their attempts to isolate the vitamin from lemon juice or cabbage juice it became increasingly evident that oxidation rapidly destroyed the potency of the vitamin.

This was further confirmed by studies of the methods of preserving antiscorbutic foodstuffs. The commercial canning process (Eddy and Kohman, 1924-25) was found to owe its protective action against loss of vitamin C potency to control of oxidation. In 1922, Zilva showed that decitrated lemon juice lost 80% of its potency in one-half hour if made N/20 alkaline and exposed to air at room temperature. No loss of potency occurred if air was excluded. That oxidation is the destructive process was confirmed by Kennedy in Sherman's laboratory in 1926.

These and many other similar studies proved that vita-

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min C (the antiscorbutic factor) is an easily oxidized compound and one whose oxidation is notably reduced by the maintenance of an acid reaction. For reviews of these studies see King (1936, 1938).

Progress toward isolation of the vitamin was delayed from 1916 to 1920 by the infection theory of scurvy origin. In 1916 Jackson and Moore recovered from guinea pigs, which had been made scorbutic by a diet of oats and milk, a diplococcus which they suggested might be the etiological factor. Since oats and milk were known to provide a complete diet for rats, Jackson's results seemed to exclude diet as a causative factor.

The following year McCollum and Pitz (1917) confirmed the production of scurvy in guinea pigs by feeding diets of oats and milk and supported Jackson's theory. McCollum has described their attitude at the time in these words:

"They found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives."

Examination of their oats- and milk-fed guinea pigs showed the caecum distended with impacted feces. They therefore felt that this also confirmed the infection theory.

Shortly after, however, Chick and Hume (1919) and Cohen and Mendel (1918) produced evidence that reconfirmed the probable dietary origin of the disease. Chick and Hume showed that milk was a far poorer protective against scurvy than had been assumed; and Cohen and Mendel, by feeding a superior diet, were able to produce scurvy in a guinea pig without developing impacted feces or producing caecal lesions.

A few years later Parsons (1924) furnished the final

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explanation of McCollum's inconsistent results. Parsons found that while oats and milk constituted a protective diet for rats, this was caused, not by the vitamin content of milk but by the complete immunity of the rat to scurvy. Parsons found that the livers of rats reared on a diet deficient in the antiscorbutic factor contained significant amounts of this factor, and that rats, unlike guinea pigs and man, synthesize enough of the vitamin for their needs. This was confirmed by further studies by Parsons and Hutton (1924) and by Lepkovsky and Nelson (1924). The false trail was therefore abandoned and search for the antiscorbutic substance resumed.

A specially effective aid to this search was found in significant contributions made by Tillmans and Hirsch (1932). These chemists, at Frankfort, Germany, had occasion to distinguish between fresh and stale and between true and artificial fruit juices. They found that distinction could be made by using an oxidation-reduction indicator known as phenol-indophenol. Fresh juice gave a strong reaction with the reagent, stale juices a lesser reaction. Artificial juices did not affect the indicator.

Zilva (1935) agreed that antiscorbutic juices bleach phenol-indophenol, and found that he could determine the reducing capacity of antiscorbutic substances by means of the indicator, but that the results did not always parallel estimation of vitamin C by animal tests. From these comparisons Zilva reached the conclusion that:

"Vitamin C itself did not reduce indophenol, but the decolorization of the indicator was due to a bleaching substance closely associated with the active principle, which tended to prevent oxidation."

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Tillmans took exception to this view of Zilva's, and contended that it was the vitamin itself which bleached the indicator. In advancing this view he relied on studies proving that the reduction of the indicator was due to vitamin C itself, that the indicator measured the concentration of the vitamin and its physiological potency, and that the vitamin might be hexuronic acid. He held that the oxidation of the vitamin was reversible and that in the first stage of oxidation the vitamin was more likely to be destroyed by further oxidation than in its original reduced form. Though Tillmans did not know of it at the time, Szent Gyorgyi (1933) had already demonstrated the reversible oxidation of hexuronic acid.

This long series of studies therefore confirmed Funk's 1914 prediction that scurvy would prove to be a vitamin-deficiency disease.

Scurvy

The general subject of scurvy has been well covered in Hess' (1920) monograph. The relation of ascorbic acid to the prevention of the disease is now gradually clarifying, with the availability of the pure vitamin for study.

Dalldorf (1940) points out that, although lack of vitamin C is a specific cause of scurvy, there are other factors controlling the development of the disease, and for that reason there are occasional discrepancies between the estimates for vitamin C need and response of individuals to such dosage. Elmby and Warburg (1937), for example, noted that of 29 cases of mild scurvy, 26 responded within 10 days to 300 mg. of ascorbic acid given orally; but three showed

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no improvement and still failed to respond to 300 mg. given parenterally. They did, however, respond to the juice of 10 lemons given orally. It may well be that certain manifestations we have listed as scurvy symptoms require other vitamins such as vitamin P of Szent Gyorgyi (1936), which is discussed further in Chapter Ten.

The characteristics of mild scurvy in infants have been tabulated by Frohlich (1912) as follows: dystrophy, anorexia, anemia, occasional slight edema, cessation of gain in weight or loss of weight, susceptibility to infection, intestinal disturbance, and now and then hematuria. In acute scurvy the most characteristic indication is hemorrhage and hemorrhagic diathesis, or tendency to bleeding. Scurvy is shown to have an asymptomatic stage which precedes characteristic symptoms. One problem of the clinician today is to be able to diagnose this stage and prevent further development of the disease.

Pathology of Scurvy

It will be recalled that lack of vitamin A resulted in hardening of the epithelial tissues, and that something was apparently needed *within the cell* for its normal form and behavior. In 1919 Aschoff and Koch advanced the view that ascorbutic hemorrhage and other scorbutic tissue changes were due to lack of something essential to the normal function and behavior of intercellular materials, especially those associated with the connective or mesenchymal tissues.

Dalldorf (1938) points out that the primary morphologic effect of vitamin C deficiency does occur in the intercellular substances of certain mesenchymal derivatives. If we consider

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the simplest prototype of these tissues, loose connective tissue, we observe the following. Under normal conditions the type cell (the fibroblast) lies in an amorphous ground substance within which fibrils are formed, which in turn become cemented together into wavy bands of collagen like the setting of a gel. In guinea pigs depleted of vitamin C the fibroblasts are present just as in healthy pigs, but fibrils and collagen fail to form. With adequate dosage of vitamin C these intercellular substances appear within 18 hours.

In bone, the functioning cells are osteoblasts and the intercellular substance is osteoid tissue. In the teeth the functioning cells are odontoblasts and the intercellular substance is dentine. In both bones and teeth, lack of adequate vitamin C affects the character of the ground, or intercellular, substance and supplying vitamin C quickly restores normality to the material.

There is, then, no question that vitamin C deficiency affects the formation of normal intercellular substance. The controversial point is whether it produces this effect by supplying something the cell needs for its manufacture or control, or whether deficiency of the vitamin produces an effect by interference with the metabolism of the fibroblasts, osteoblasts and odontoblasts themselves. Opinions differ on this point. Fish and Harris (1938) and Hojer (1924) believe that lack of C produces atrophy of the cells themselves. Wolbach and Howe (1926) incline to the view that it supplies something the ground substances need, something, for example, like the pectin the housewife uses to insure the "setting" of her jellies.

Regardless of *how* the C deficiency effect is produced, examination of the character of the ground substance permits

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the pathologist to determine histologically the presence or absence of vitamin C deficiency.

Vitamin C Deficiency Effects Definitely Due to Modification of Intercellular Substance

Scorbutic Bleeding. Hess (1914), by applying a tourniquet and thus subjecting capillaries to increased pressure, found that in cases of scurvy this caused greater bleeding than in normal individuals. In brief, the capillaries of scorbutic individuals tended to leak more readily under increased pressure than those of normal individuals. Göthlin (1930) in Sweden developed a test for capillary dietary deficiency. In this country Dalldorf (1933 and 1935) perfected a capillary resistance manometer for the same purpose and has made a rather extensive study of the behavior of scorbutics with this instrument. Hess (1914) warned that there are other factors than C deficiency involved in tendency to bleeding; and the discovery of vitamins K and P has emphasized the importance of this warning against assuming that a low capillary resistance necessarily proves vitamin C deficiency.

Discussing his own developments, Dalldorf (1940) has made the following comment:

“A thorough trial of the capillary test as a measure of vitamin C deficiency in groups of children has been made by Roberts, Blair and Bailey (1939). Their report is recommended both as being a thorough trial of the test and a good review of the experiments of others. A distinct statistically significant correlation was found between season, capillary resistance and ascorbic acid intake. The differences between the children on an institutional diet and those receiving supplements of vitamin C are shown below:

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Group	No Fragility Present	Fragility Present
Control group	32%	26%
With vitamin C addendum	53%	5%

"The virtue of the capillary test is that it is a measure of scurvy and capillary fragility due to vitamin C depletion as identified by a test dose of vitamin C and, followed by observations of the resistance, is *prima facie* evidence of a pathological degree of depletion. This, the chemical tests of blood and urine content, supply only by inference. There is no reason to believe that it is precise or uniform to any greater degree than other measurements and much of the criticism of it has come from individuals who have looked for a degree of precision that the test lacks."

When lack of vitamin C is shown to produce capillary fragility and the proof of this is correction of that fragility by vitamin C dosage, how is the resistance to pressure effected?

The endothelium, or lining membrane, of the capillaries is believed to be fused together by a cementing substance. The capillary is also surrounded by connective tissue and the endothelium is ensheathed with collagenous fiber. It is not yet clearly established whether it is the endothelium fusing material that is lacking, or failure to form collagen on the part of connective tissue cells forming the sheath. Dalldorf inclines to the view that it is the intercellular substance that is lacking and not loss of the ability of connective tissue cells to proliferate the leak-preventing substance.

Scorbutic Bone Changes. Scurvy often produces lesions at the joining of the ribs, called the costochondral junction, and at the ends of certain bones. At these regions there is often a cessation of bone growth and replacement with collagen-poor connective tissue in which may be embedded fragments of densely calcified cartilage. The cells in these

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regions are frequently osteoblasts which have reverted to the primitive fibroblasts. The condition suggests that in the absence of vitamin C the osteoblasts, being unable to form osteoid or bony tissue, revert to their primitive connective tissue form and try to set up a fibrous union. The zone where this development occurs is spoken of as the "gerüstmark", or framework marrow. This gerüstmark shows up in x-ray and is a means of diagnosing vitamin C deficiency. This sort of bone lesion is often accompanied by hemorrhages which may be just under the periosteum or in the bone itself.

Some of these changes in bone are strikingly like the changes that occur in rickets, and it is often difficult to determine whether the deficiency is of vitamin C or vitamin D, especially when scurvy is complicated with rickets, as often happens in infants. The periosteum shows weakening of attachment as well as hemorrhagic condition which again indicates a deficiency of something required by the connective tissue. The bone, then, like the capillaries, responds to vitamin C deficiency by a failure in or a faulty production of intercellular material.

Teeth. In the teeth the dentine or bony material filling the space between the root canal and the enamel is a product of specialized cells called odontoblasts. In the guinea pig, deprivation or reduction in adequate supply of vitamin C has been shown to produce the following changes: within four or five days the odontoblasts shorten and become separated from the dentine by a fluid zone. If the deprivation is complete and is maintained until the death of the animal, usually about three weeks, these odontoblasts actually revert to a spindle-form and are indistinguishable from the connective tissue cells in the pulp of the tooth. Simultaneously the Tomes

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canals, which are seen as striations in the tooth, widen appreciably, bringing about a porosity of the dentine. At the same time the teeth cease to grow.

If the deficiency is partial and prolonged for several months, the odontoblasts continue to secrete, but produce instead of dentine a substance resembling bone, which gradually fills the pulp canal. Addition of ascorbic acid brings a prompt reaction, and cells which have been affected by the C deficiency become restored to original appearance and function. These changes in the dentine and dentine-forming cells are usually accompanied by increased blood in the root pulp and tendency to hemorrhage. The dental lesions commence in the crown of the tooth and proceed toward the root.

It is evident that if lack of vitamin C can produce such marked changes in the formation of dentine it is especially important that when teeth are forming in infancy and childhood, especial attention be given to adequacy of vitamin C to prevent a faulty supporting structure for the enamel.

Dental investigators today distinguish between two types of caries, one of which they call true caries and the other fissure caries. By true caries they mean the production of a hole in intact enamel; by fissure caries, they mean tooth decay which is initiated by bacteria lodging in cracks that have been produced mechanically in the enamel. Such cracks or fissures are obviously more apt to occur in biting if the underlying dentine is not properly constructed.

Hanke (1933) reported that marked benefit in prevention of dental caries is derived from generous dosage with orange juice, and also that vitamin C-rich citrus fruit juices were of value in protection against gingivitis (gum inflam-

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mation) and periodontoclasia (pyorrhea). There has been very extensive research conducted in the past few years to determine causes of dental caries. There is general agreement today that these causes are multiple, and that vitamin C alone will not prevent true caries in teeth which are already formed, though it may be a factor in such protection. Gingivitis, or gum inflammation, may result from hemorrhage caused by C deficiency. Nasal bleeding is a common accompaniment of such condition and due to the same cause, lack of adequate amount of vitamin C in the diet.

There has been recent evidence of a direct relation between vitamin C and pyorrhea. The loosening of teeth in scurvy by both man and animals has been frequently observed. In 1937, Boyle, Bessey and Wolbach pointed out that, besides striking alterations in tooth pulp and dentine in vitamin C deficiency, there may be changes in the soft and calcified tissues around the tooth (peridental tissues). They suggested that there may be two types of pyorrhea, a local inflammatory disease and a systemic process causing diffuse atrophy of the alveolar bone.

The systemic type as it occurs in infantile scurvy is similar to the conditions found in guinea pigs on a C-deficient diet; and in a limited number of patients with this type of pyorrhea they noted a correlation between low blood ascorbic acid values and rarefaction of the alveolar bone. They suggest that a low vitamin C intake may therefore be an important factor in the production of this type of systemic pyorrhea. They reported that 23 cases showed low vitamin C in the blood and mouth tissues and were improved by vitamin C supplement. They used 150-200 mg. daily for treating this type of pyorrhea.

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Abt and Farmer (1938) summarize the tooth situation as follows:

“Although there is still a dearth of exact knowledge of vitamin C in its relation to dental and gingival diseases in man, there is a general unanimity of opinion that an adequate intake of vitamin C is necessary for normal tooth growth and tooth structure, and the maintenance of healthy gums in man.”

Pigmentation. There is a condition known as Addison's disease which is characterized by a pigmentation of the skin. Several observers have noted a reduction in this pigmentation in such cases following administration of vitamin C (Wilkinson, 1936; Schroeder, 1936; Cornbleet, 1937; Abt and Farmer, 1938).

The adrenals have been known for some time to have a high ascorbic acid content of both the cortex and the medulla. The usual treatment of Addison's disease has been with adrenal cortex extract. This fact and the above observations have suggested that both the vitamin C and the adrenal cortex hormone are necessary for prevention of Addison's disease, the vitamin C being one of the factors controlling the intercellular substances which form the pigment. To date those who have studied the effect of vitamin C on Addison's disease agree that it does not improve the symptoms of that disease and that its effect is confined to pigmentation.

Indications of Vitamin C Deficiency. There is strong evidence to indicate that what used to be known as “country” rheumatisms, namely stiff joints that developed in long winters on diets lacking in greenstuff, and which cleared up promptly with the eating of fresh fruits and vegetables in the spring, were actually hemorrhagic joint conditions pro-

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duced by vitamin C deficiency. In brief, whenever one finds evidence of hemorrhage in tissue or organs, that sign in itself is an indication that the cause may possibly be lack of adequate amount of vitamin C in the diet. The sign itself, however, is not a conclusive proof of present deficiency until confirmed by a positive response to vitamin C addendum.

Measurement of Vitamin C Deficiency. We have already noted that measurement of vitamin C deficiency is possible by use of the capillary resistance machine. During the past few years, two other methods of estimating it have come into common use. Both of them are based on what is called the test dose method. In principle this method consists of feeding a high dosage of vitamin C, collecting the urine for twenty-four hours and determining the output in that period. If that output equals fifty per cent or more of the ingested vitamin the test is taken to indicate saturation of the body tissues; if less than fifty per cent a vitamin C deficiency is indicated.

Abt and Farmer (1936, 1937) have worked out a micro-chemical test for vitamin C in blood. This test has been rather extensively developed in quite a large number of laboratories. It appears that a vitamin C distribution in the blood of 1 mg. per 100 cc. or more indicates complete protection against scurvy; also that a blood concentration of 0.5 mg. or less indicates definite scurvy, and that between 0.5 and 1.0 mg. % is a borderline state.

Whether the test as worked out by Abt and Farmer is one hundred per cent correct for quantitative estimation of the blood C content, and whether the figures given above for blood content truly represent the saturation conditions in

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the tissues is still undetermined. It is also true that blood values lower than 0.5 mg.% are not always accompanied by the diagnostic symptoms of scurvy. However, as in the case of blood vitamin A and carotene tests, relative results by contrast give us important diagnostic signs which help toward a satisfactory diagnosis of vitamin C deficiency.

Such tests are now being extensively used as index to the need of vitamin therapy in special cases. They form the present basis for recommendations as to daily requirement and for treatment of specific diseases.

When intercellular substance is deranged, the diagnosis of C deficiency becomes definitely possible. When, however, we have a subclinical condition of scurvy in which the pathologic signs have as yet failed to develop, the blood test may be indicative of tendencies toward scurvy. In the following pages we have outlined several disease conditions which appear to be associated with vitamin C deficiency as determined either by assays of the food intake or by blood tests. It is impossible at present to state whether the disease conditions described were caused as a result of the lack of vitamin C for building materials or lack of vitamin C for the control of the connective tissue cells which produce intercellular substance.

Blood Changes

In addition to the effect of vitamin C on capillary fragility there is evidence that this deficiency may also affect the constituents of the blood. Terazawa (1937) claims that vitamin C accelerated the coagulation time of rabbit's blood,

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increased the blood platelets, the fibrinogen and the thrombin. Cotti (1936), after giving human subjects intravenously 200 mg. of vitamin C, stated that the coagulation time was shortened in hemorrhagics and prolonged in normal persons; that vitamin C promotes the activity of thrombin when it is diminished and inhibits it when it is normal in amount, and that it does not affect fibrinogen and prothrombin. He suggests that this effect is due to the reducing action of vitamin C.

Schneider and Widman (1935) have reported that the therapeutic administration of vitamin C may produce a decrease in globulin and increase in albumin content of blood, accompanied by reduction in the sedimentation time of the red cells. Stephens and Hawley (1936) noted that the leukocytes carry a greater quantity of vitamin C than either the plasma or the red cells; and it has been suggested that vitamin C has a direct relation to the number of circulating leukocytes (Cuttle, 1938). Eufinger (1936) has reported a case of myeloid leukemia brought back to normal by injection of 2000 mg. of ascorbic acid. In scurvy there is usually a moderate degree of anemia of the microcytic type, and it has been shown that vitamin C is beneficial in some of these cases when iron and liver extracts were ineffective (Minot, 1935). Wolbach (1937) states that in long-continued partial vitamin C deficiency in guinea pigs, large regions of the bone marrow became devoid of blood-forming cells and are replaced by a homogeneous, starch-like material.

There is, then, evidence that vitamin C may be helpful in combating nutritional anemia but no definite evidence of its actually helping the building of hemoglobin.

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Vitamin C and Immunology

In 1935 King and Menten reported that a guinea pig's resistance to diphtheria toxin is increased by vitamin C administration. Previous to their report, Sulzberger and Oser (1934) had demonstrated that large doses of vitamin C diminished and inhibited the susceptibility of guinea pig's skin to experimental sensitization with neoarsphenamine, and that the dose necessary to achieve this effect was higher than the minimal dose necessary to protect against scurvy.

Jungebluth and Zwemer (1935) claimed that diphtheria toxin could be inactivated *in vitro* by this vitamin. On the other hand, Pakter and Schick (1938) could produce no effect on diphtheria toxin in a series of children known to react positively to the Schick test. They believed the action of the vitamin on the toxin *in vitro* is not specific, perhaps simply due to pH change or to some oxidation-reduction effect. In reviewing these experiments King (1938) states, however, that:

"A number of papers have added further evidence in support of the viewpoint that vitamin C is of major importance in the detoxification process."

Commenting on a paper by Kaiser and Slavin (1938), the *Journal of the American Medical Association* says editorially:

"These results suggest that streptococci are less likely to be found in tonsils when the vitamin C values of blood are high and that when present in such cases they are seldom virulent. While the vitamin C content of the blood is probably determined by the amount taken in with certain foods, the daily ingestion of a reasonable amount of orange juice apparently does not insure a high level of vitamin C in the blood in all instances. When generous amounts of vitamin C are taken daily in the form of fruit juices, the vitamin C blood levels

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are uniformly high or in the average zone. The definite determination that there are fewer streptococci present in the tonsils of children with an average or better vitamin C content suggests an inhibitory relation. The desirability of supplying children with more than the minimum amount of vitamin C in their diets is obvious."

Claims have been made of the value of vitamin C in combating infections such as diphtheria, rheumatic fever, tuberculosis, etc., and the subject needs further study.

King (1939) states that injections of toxins or other toxic materials may cause a marked depletion of vitamin C from the tissue, and that animals in general whose tissues are moderately or severely depleted of their vitamin C reserves are subject to greater injury by toxin injections.

Ecker (1938) has reported extensive studies on the relation of vitamin C to serum complement. These studies showed a definite correlation between the amount of vitamin C and guinea pig serum and the complement titer; and these observations have been confirmed by other laboratories.

The complement-fixation test has already wide application, as in the Wassermann test for syphilis; and the relation of C to complement behavior is an important contribution to the understanding of immunological reactions.

The mechanism by which C produces this effect appears related to its reducing capacity. Eckers supports this view by demonstration that chemical reductors such as hydrogen sulfide, sodium thiosulphate and glutathione can activate complement.

Faulkner and Taylor (1937) have reported that serum ascorbic acid levels in patients with infections are usually well below the values encountered in normal individuals. This has been confirmed by Wright. Heise and Martin (1937)

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have reported that in pulmonary tuberculosis the activity and the extent of the disease show a certain correlation with the degree of vitamin C deficiency. They state that to maintain a normal rate of excretion in the presence of tuberculosis the patient requires from 55 to 100 mg. daily.

There are, then, a good many observations indicating that the presence of infection creates an increased demand for vitamin C, that vitamin C may be reduced by destruction through the action of infecting organisms, and that it may be a factor in controlling the immune bodies used to combat infections. But, as stated above, the exact way in which the vitamin functions is still undetermined. Ecker does not believe that vitamin C itself is complement, but essential to the activity of complement.

Collagen is an intercellular substance. It is important for segregating infectious organisms as, for example, in tuberculosis. Since vitamin C is a controlling factor in collagen formation, part of its relation to the tubercular patient's requirement may be connected with this activity.

Other Correlations of Vitamin C Deficiency and Pathology

It has been both claimed and denied that vitamin C has an antagonistic action to thyroid activity similar to that reported for vitamin A. It has been reported to have a detoxicating and protective function in the gastro-intestinal wall. Einhauser (1936) and Pescarmona (1937) claim that it decreases the elimination of uric acid and urea. It has also been reported (Negrie, 1937) to aid in the breakdown of oxybutyric acid and hence to reduce the tendency to ketosis in diabetics. It has also been suggested (Pfleger, 1937) that

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it has some action on blood and urinary sugar in diabetes.

The vitamin has been reported to be of value in the treatment of bronchial asthma, whooping cough, and certain nervous disorders. Reiss (1937) has noted the reduced excretion of vitamin C even though the dietary intake was within normal range in cases of psoriasis, and believes that the effect is due either to destruction of the vitamin by the infective agents causing the psoriasis, or to disturbed skin metabolism which creates a demand for more of the vitamin. Rosenberg (1938) has claimed that vitamin C has a value in prevention of urticarial lesions.

The fact that vitamin C can be reversibly oxidized tempts one to consider it as having a part in cellular oxidation and reduction procedures. We know that the requirement is definitely increased with heightened metabolism, as in fevers and infectious diseases.

Hawley (1936) claims that the amount excreted is changed by altering acid-base balance of the food intake. Sigal and King (1937) have suggested that it influences carbohydrate metabolism. They show that guinea pigs depleted of vitamin C in successive stages showed a corresponding rise in blood sugar and distinctly lowered sugar tolerance. After 10 days' depletion, readministration of ascorbic acid brought return of blood to normal sugar value in 15 days, but other vitamins failed to produce this effect.

Chakraborty and Roy (1938) measured the urinary excretion of vitamin C of two human subjects on varying diets. They showed depletion of C output in high carbohydrate intake, and increase on high meat and high fat diets. These results of course suggest that the carbohydrate creates de-

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mand for C in connection with its metabolism, a demand not made by proteins or fats. Nevertheless, King (1938) states that at the present time it is impossible to indicate with certainty any specific relationship between vitamin C and the enzymes in animal tissues, although activating and inhibiting effects have been reported and may be significant. That vitamin C itself acts as a major respiratory catalyst, however, is contradicted by the fact that depleted tissues, when measured for oxygen uptake capacity, show no decrease in such capacity over the normal. Neither is there rise in oxygen consumption when ascorbate is added to the depleted tissues. In brief, there is at present no positive evidence of ascorbic acid acting as the prosthetic group in any enzyme compound in animal tissues.

To what extent these various effects that have been undoubtedly observed are due directly to vitamin C deficiency or to malnutrition in general associated with vitamin C deficiency requires much further study. In many cases the enhancement of the diet with vitamin C has brought improvement, not because that deficiency was a primary cause of the disturbance but because supplying the tissues with their needed quota restored them to a condition in which they could fight against disease. We have seen examples of this in the correction of bleeding conditions associated with colitis in peptic ulcer.

Vitamin C Requirement

In the discussion of methods for measuring vitamin C deficiency it has been noted that one procedure now exten-

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sively practiced is to give the patient a test dose and note the extent of elimination in the succeeding 24 hours in the urine. Farmer (1939) has shown that fecal excretion is relatively small and that urinary excretion is the main path for removal of vitamin C from the body.

On the basis of test doses certain recommendations have been made as to vitamin C needs based on such saturation figures. For example, Abbasy *et al.* (1935) suggested that a day-to-day excretion of 10 mg. of ascorbic acid in the urine indicates borderline between adequacy and deficiency, and 40 mg. a liberal intake. Later (Harris *et al.*, 1936) suggested that if a subject excretes less than 13 mg. of ascorbic acid per day and fails to respond to a test dose of 700 mg. per 140 lbs. of body weight, the diet contains less than the minimum quantity of C required. Van Eekelen (1937) set a higher figure, calling for daily excretion of 40 mg. as indicating body saturation.

Since the urinary output varies with vitamin C intake and the fecal output is relatively insignificant, the test by this method for saturation is now believed to indicate adequacy in the diet, if the body excretes 50 to 70% of a large test dose in the subsequent 24 hours.

The blood test is now generally interpreted as follows: If the blood plasma content of ascorbic acid falls below 0.5 mg. per 100 cc. the individual may be considered in a potentially scorbutic condition. If the blood content is 1 mg. per 100 cc. or more, the intake and absorption may be considered normal and adequate. Values between 0.5 and 1 mg.% are borderline cases suggesting need for increased vitamin C. However, the fact remains that, as previously

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stated, many cases showing lower values of blood C fail to show detectable signs of scurvy. The question in such cases is whether those low figures truly represent evidence of saturation or whether there are symptoms of sub-clinical scurvy still unrecognized by the diagnostician.

Correlation between test dose, urine and fecal elimination is illustrated in the following table supplied through the courtesy of Dr. Farmer:

Table 14.
(After C. J. Farmer, 1939)

Sub- ject	Before Taking Test Dose—			After 1000 mg. Ascorbic Acid Test Dose				
	Blood Plasma Con- tent (mg. %)	Daily Urinary Excretion (mg.)	Daily Fecal Excretion (mg.)	Blood Plasma Content (mg. %)	24 Hour Urinary Excre- tion (mg.)	Test Dose in Urine (%)	24 Hour Fecal Excretion (mg.)	Test Dose in Feces (%)
1.	0.32	23.6	5.76	1.20	335.1	34	7.32	0.7
2.	0.48	25.9	2.88	1.84	959.0	96	30.10	3.0
3.	0.60	59.9	3.45	1.56	443.9	44	11.29	1.1
4.	0.72	27.2	3.81	2.20	645.9	65	12.76	1.3
5.	0.80	21.6	0.16	1.24	387.9	39	2.92	0.2
6.	1.00	33.6	7.87	1.96	605.5	61	16.91	1.7

These results show that the correlation between urinary excretion and blood data is not perfect. They indicate that we need further study before we can absolutely rely on these tests to criticize diet intake or report scorbutic state. However, they are a beginning in the steps necessary to reach true conclusions regarding the effect of vitamin C deficiency.

Incidentally, the fact that we are also as yet uncertain whether the quantitative chemical procedures we follow to obtain these results are themselves one hundred per cent accurate indicates need for further study of the methods themselves. The availability of pure ascorbic acid is aiding materially in such research.

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Bibliography

- Abbasy, M. A., Harris, L. J., Roy, S. N., and Marack, J. R., *Lancet*, 2, 1399 (1935). Diagnosis of Vitamin C Subnutrition by Urine Analysis.
- Abt, A. F., and Farmer, C. J., *J. Pediatr.*, 8, 1 (1936); *Am. J. Dis. Child.*, 54, 682 (1937). Normal Cevitamic Acid Determinations in Blood Plasma.
- Abt, A. F., and Farmer, C. J., *J. Amer. Med. Assn.*, 111, 1555 (1938). Vitamin C; Pharmacology and Therapeutics.
- Aschoff, L., and Koch, W., "Skorbut," Jena, G. Fischer, 1919.
- Bezssonoff, N., *Bull. Soc. Chim. Biol.*, 9, 568 (1927). Suggestion That Vitamin C Has Tripartite Nature Consisting of a Phenol, a Reducing Sugar, an Organic Acid. *Bull. Soc. Chim. Biol.*, 11, 294 (1929). Two Notes on Color Reactions of Vitamins.
- Boyle, P. E., Bessey, O. A., and Wolbach, S. B., *Proc. Soc. Exp. B. & M.*, 36, 733 (1937). Exper. Alveolar Bone Atrophy Produced by Ascorbic Acid Deficiency.
- Chakraborty, R. K., and Roy, G. K., *J. Amer. Med. Assn. Review*, Rise in C on High Meat Diets and Lowering with Carbohydrate Feeding.
- Chick, H., and Hume, E., *Trans. Soc. Trop. Med. Hyg.*, 10, 179 (1916-17); *Proc. Roy. Soc. London (B)*, 90, 44 (1917-19). Distinction Between Beri-beri and Scurvy Factor.
- Chin, H., and Farmer, C. J., *Proc. Soc. Exp. B. & M.*, 41, 370 (1939). Fecal Excretion of Vitamin C.
- Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 35, 425 (1918). Cause of Scurvy Deficiency.
- Cornbleet, T., *Arch. Dermat. and Syph.*, 35, 471 (1937). Vitamin C and Pigment.
- Cotti, L., *Hematologica Arch. Pavia.*, 17, 483 (1936). Further Research on the Influence of Vitamin C on Blood Coagulation.
- Cuttle, T. D., *Quart. J. Med.*, 7, 575 (1938). Observations on the Relation of Leucocytosis to Ascorbic Acid Requirements.
- Dalldorf, G. N., *J. Exper. Med.*, 53, 289 (1931). Criterion of Hemorrhagic Diathesis in Experimental Scurvy. *Am. J. Dis. Child.*, 46, 794 (1933). A Sensitive Test for Subclinical Scurvy in Man. *J. Amer. Med. Assn.*, 111, 1376 (1938). The Pathology of Vitamin C Deficiency. *Personal Communication*, 1940. Capillary Resistance Measurements.

VITAMIN C

- Dalldorf, G. N., and Russell, H., *J. Amer. Med. Assn.*, 104, 1701 (1935). The Effect of Cevitamic Acid Injections on Capillary Resistance.
- Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 34, 19 (1938). Ascorbic Acid and Complement Function.
- Ecker, E. E., Pillemer, L., Griffiths, J. J., and Schwartz, W. P., *J. Amer. Med. Assn.*, 112, 1449 (1939). Complement and Ascorbic Acid in Scurvy.
- Eddy, W. H., and Kohman, E. F., *J. Ind. & Eng. Chem.*, 16, 52, 53 (1924). Vitamin C in Canned Foods.
- Van Eekelen, M., *Biochem. J.*, 30, 2291 (1936). Adult Requirement of Vitamin C.
- Einhauser, M., *Ztschr. f. d. ges. exper. Med. Berlin*, 98, 461 (1936). C Vitamin and Gastro-Enteritis.
- Elmby, A., and Warburg, E., *Lancet*, 2, 1353 (1937). Inadequacy of Synthetic Vitamin C as an Antiscorbutic Agent.
- Eufinger, H., and Gaetgens, G., *Klin. Wochenschr.*, 15, 150 (1936). Effect of Vitamin C on White Corpuscle Content of Blood.
- Farmer, C. J., and Abt, A. F., *Proc. Soc. Exp. B. & M.*, 34, 146 (1936). Determination of Reduced Ascorbic Acid in Small Amounts of Blood.
- Faulkner, J. M., and Taylor, F. H. L., *Ann. Int. Med.*, 10, 1867 (1937). C Blood Level in Infected Cases.
- Fish, E. W., and Harris, L. J., *Brit. Dent. J.*, 58, 3 (1935). The Effects of Vitamin C Deficiency on Tooth Structure in Guinea Pigs.
- Frohlich, T., *Z. Hyg. Infektionskr.*, 72, 155 (1912). Infantile Symptoms of Scurvy.
- Gothlin, G. F., *J. Lab. and Clin. Med.*, 18, 484 (1933). Outline of a Method for the Determination of Strength of Skin Capillaries.
- Hanke, T., "Diet and Dental Health," Univ. Chicago Press, 1933.
- Harris, L. J., and Ray, S. N., *Biochem. J.*, 26, 580 (1933). Normal Urinary Excretion of Vitamin C Equals 15-30 Mg.
- Harris, L. J., Abbasy, M. A., Yudkin, J., and Kelly, S., *Lancet*, 1, 1488 (1936). Vitamins in Human Nutrition.
- Hawley, E. E., Frazer, J. P., Button, L. L., and Stephens, D. J., *J. Nutr.*, 12, 215 (1936). Effect of Acid Base on Urinary Content Ascorbic Acid.
- Hawley, E. E., Stephens, D. J., and Anderson, G., *J. Nutr.*, 11, 135 (1936). Normal Urinary Excretion of Vitamin C.
- Haworth, W. N., and Hirst, E. L., *J. Soc. Chem. Ind.*, 52, 221, 481 (1933). Constitution of Ascorbic Acid.

WHAT ARE THE VITAMINS?

- Heise, F. H., and Martin, G. J., *Proc. Soc. Exp. B. & M.*, 34, 642 (1936); *Am. J. Dig. Dis. & Nutr.*, 4, 368 (1937). Ascorbic Acid Metabolism and Nutrition in Pulmonary Tuberculosis.
- Hess, A. F., "Monograph on Scurvy," Lippincott, 1920.
- Hess, A. F., and Fish, M., *Am. J. Dis. Child.*, 8, 385 (1914). Infantile Scurvy: The Blood, the Blood Vessels and the Diet.
- Hojer, A., *Acta Pediat.*, 3, 8 (1924). Tooth Changes in Scurvy.
- Holst, A., and Frohlich, T., *J. Hygiene*, 7, 634 (1907). On the Etiology of Scurvy.
- Jackson, L., and Moore, J. S., *J. Infect. Dis.*, 19, 478 (1916). Studies on Experimental Scurvy in Guinea Pigs.
- Jungebluth, C. W., and Zwemer, R. L., *Proc. Soc. Exp. B. and M.*, 32, 1229 (1935). Inactivation of Diphtheria Toxin in Vivo and Vitro by Crystalline Vitamin C.
- Kaiser, A. D., and Slavin, B., *J. Pediatr.*, 13, 322 (1938). The Incidence of Hemolytic Streptococci in the Tonsils of Children as Related to the Vitamin C Content of Tonsils and Blood.
- Kenny, C. L., Diss. Columbia Univ., 1926. A Study of the Thermolability of Vitamin C.
- King, C. G., *Physiol. Rev.*, 16, 238 (1936). Vitamin C. *J. Amer. Med. Assn.*, 111, 1098 (1938). The Physiology of Vitamin C. *J. Amer. Med. Assn.*, 111, 1462 (1938). The Chemistry of Vitamin C. *Ann. Rev. Biochemistry* 392 (1939). The Water-Soluble Vitamins.
- King, C. G., and Menten, M. L., *J. Nutr.*, 10, 129 (1935). Influence of Vitamin C Level Upon Resistance to Diphtheria Toxin.
- Lepkovsky, S., and Nelson, M. T., *J. Biol. Chem.*, 59, 91 (1924). Observations on the Persistence of Vitamin C in the Livers of Rats on a Scorbutic Ration.
- Lind, J., "Treatise on Scurvy," London, 1757.
- McCollum, E. V., and Pitz, W., *J. Biol. Chem.*, 31, 229 (1917). The Vitamin Hypothesis and Deficiency Diseases.
- Minot, G. R., *J. Amer. Med. Assn.*, 105, 1176 (1935). Anemias of Nutritional Deficiency.
- Negri, C., *Giornal di clinica med. Parma*, 18, 485 (1937). Vitamin C and the Ketolysis of Oxybutyric Acid.
- Pakter, J., and Schick, B., *Am. J. Dis. Child.*, 55, 12 (1938). Vitamin C Not a Specific for Diphtheria Toxin Destruction.

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- Parsons, H. T., *J. Biol. Chem.*, **44**, 587 (1920). The Antiscorbutic Content of Certain Body Tissues of the Rat.
- Parsons, H. T., and Hutton, M. K., *J. Biol. Chem.*, **59**, 97 (1924). Further Observations Concerning the Antiscorbutic Requirement of the Rat.
- Pescarmona, M., and Quaglia, F., *Arch. p. l. Stud. d. Fisiopat. e Clin. d. Ric. Siena*, **5**, 247 (1937). Influence of Vitamin C on Metabolism of Uric Acid.
- Pfleger, R., and Scholl, F., *Wien Arch. F. inn. Med.*, **31**, 169 (1937). Diabetes and Vitamin C.
- Poncher, H. G., and Steubenrauch, C. H., *J. Amer. Med. Assn.*, **111**, 302 (1938). Intradermal Test for Vitamin C Deficiency.
- Reichstein, T., Grussner, A., and Oppenhauer, R., *Helv. chim. acta*, **16**, 1019 (1933). Synthesis of *d*- and *l*-ascorbic Acid.
- Reiss, F., *Chinese Med. J.*, **53**, 141 (1938). Vitamin C and Psoriasis.
- Roberts, L. J., Blair, R., Bailey, M., *J. Pediatr.*, **11**, 626 (1937). Use of Lapillary Resistance to Measure Scorbutic Tendency. See also—Roberts, L. J., Brooks, M. H., Austin, G., Blair, R., Noble, I., *J. Pediatr.*, **15**, 21 (1939).
- Rosenberg, W. A., *Arch. Dermat. and Syph.*, **37**, 1010 (1938). Vitamin C and Urticaria.
- Rotter, H., *Nature*, **139**, 717 (1937); *Wiener klin. Wochenschr.*, **51**, 193 (1938). Determination of Vitamin C in Living Organisms (Intradermal Dye Test).
- Schneider, E., and Widman, E., *Klin. Wochenschr. Berlin*, **14**, 1449 (1935). Vitamin C Affects the Globulin and Albumin Content of the Blood.
- Schroeder, H., and Einhauser, M., *Munchen med. Wochenschr.*, **83**, 923 (1936). Relation of C to Addison's Disease.
- Sigal, A., and King, C. G., *J. Pharmacol. & Exper. Therapy*, **61**, 1 (1937). Influence of C Deficiency on Resistance to Diphtheria Toxin.
- Smith, F. L., and King, C. G., *J. Biol. Chem.*, **94**, 491 (1931). Relation of Vitamin C to Uric Acid.
- Stephens, D. J., and Hawley, E. E., *J. Biol. Chem.*, **15**, 653 (1936). Partition of Reduced Ascorbic Acid in Blood.
- Sulzberger, M. B., and Oser, B. L., *Proc. Soc. Exp. B. & M.*, **32**, 716 (1935). Ascorbic Acid and Sensitization to Arsphenamine.
- Svirbely, J. L., and Szent Gyorgyi, A., *Biochem. J.*, **26**, 865 (1932); *Biochem J.*, **27**, 279 (1933). Hexuronic Acid as the Antiscorbutic Factor and the Chemical Nature of Vitamin C.
- Szent Gyorgyi, A., *Biochem. J.*, **22**, 1387 (1928). Observations on the Function

WHAT ARE THE VITAMINS?

of Peroxidase Systems and the Chemistry of the Adrenal Cortex. Description of a New Carbohydrate Derivative. "Flexner Lectures," Series 6, 1939. Williams and Wilkins Co. Biological Oxidations.

Szent Gyorgyi, A., and Haworth, W. N., *Nature*, 131, 23 (1933). Hexuronic Acid as the Antiscorbutic Factor.

Terazawa, M., Takeda, K., and Mizoguchi, K., *Jap. J. Obstetrics and Gynecol. Kyoto*, 20, 550 (1937). Action of Vitamin C on Blood Coagulation Time.

Tillmans, J., Hirsch, P. and W., *Ztschr. untersuch Lebensmittel*, 60, 34, 1930; 63, 1 (1932). The Reducing Substance of Citrus Fruit Juices.

Vedder, E. B., *Mil. Surgeon*, 49, 133, 503 (1921). The Etiology of Scurvy.

Waugh, W. A., and King, C. G., *Science*, 76, 630 (1932). The Vitamin C Activity of Hexuronic Acid from Supra-Renal Glands. *Science*, 75, 357 (1932); *J. Biol. Chem.*, 97, 325 (1932). The Isolation and Identification of Vitamin C.

Wilkinson, J. F., and Ashford, C. A., *Lancet*, 2, 967 (1936). Vitamin C and Addison's Disease.

Wolbach, S. B., *Am. J. Path.*, 9, 689 (1933). Controlled Formation of Collagen and Reticulum. *J. Amer. Med. Assn.*, 108, 7 (1937). Pathologic Changes Resulting from Vitamin Deficiency.

Wolbach, S. B., and Howe, P. E., *Arch. Path. and Lab. Med.*, 1, 1 (1926). Intercellular Substances in Experimental Scorbutus.

Wortis, H., Liebman, J., and Wortis, E., *J. Amer. Med. Assn.*, 110, 1896 (1938). Vitamin C in Blood, Spinal Fluid, and Urine.

Zilva, S. S., *Biochem. J.*, 16, 42 (1922); *Biochem. J.*, 17, 410 (1923). Conditions of Inactivation of the Accessory Food Factors. *Biochem. J.*, 26, 1625 (1932). The Non-Specificity of the Phenolindophenol Reducing Capacity of Lemon Juice. *Arch. Dis. Childhood*, 10, 253 (1935). The Isolation and Identification of Vitamin C.

Zilva, S. S., and Wells, F. M., *Proc. Roy. Soc. London (B)*, 90, 505 (1919). Changes in the Teeth of Guinea Pigs Produced by a Scorbutic Diet.

CHAPTER TEN

THE FUNCTIONS OF VITAMIN P

IN HUNGARY, Szent Gyorgyi and his co-workers found (1926) that certain natural vegetable juices, notably paprika juice, showed a superiority over synthetic ascorbic acid in the prevention of capillary bleeding. Of this discovery and choice of name, Szent Gyorgyi writes as follows (1939):

"In citrus fruits we found a specially active member of this group (flavonols) present as a glucoside which up to that time had been unknown in this form. We called it with V. Bruckner 'eriodictin'. . . . In the unripe plant we find this substance in a methylated, inactive, stable form which has been known for a long time as hesperidin."

And again:

"I had a letter from an Austrian colleague who was suffering from a severe hemorrhagic diathesis (vascular type). He wanted to try ascorbic acid in his condition. Possessing at that time no sufficient quantities of crystalline ascorbic acid, I sent him a preparation of paprika that contained much ascorbic acid and the man was cured by it. Later with my friend, St. Rusznyak, we tried to produce the same therapeutic effect in similar conditions with pure ascorbic acid but we obtained no response. It was evident that the action of paprika was due to some other substance present in this plant. It would have been a hopeless job to try and find and isolate this sub-

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stance had we not had our experience with flavons. So we set out to prepare flavons, in the first place eriodictin, that can be easily injected and we found that similar pathological conditions, not previously amenable to therapy, could be cured by it with regularity. The effect had several characteristics of vitamin-action, so, tentatively, I called it 'Vitamin P' in honor of Paprika and Permeability, on which later it was found to have an influence. As yet, I have failed to demonstrate its vitamin nature by animal experiments and until such proof is given the vitamin nature of this substance is not beyond doubt."

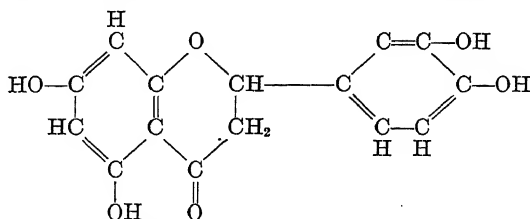
The active fraction containing Szent Gyorgyi's vitamin P as extracted from lemons has been called "citrin". It is a mixture of two dye-glucosides, the inactive glucoside, hesperidin and the physiologically active glucoside, eriodictin or eriodictyol. The eriodictyol in the extracted citrin is much less in amount than the hesperidin and can be formed from hesperidin by demethylation. Such demethylation apparently takes place during the ripening of citrus fruits, especially of the lemon, orange and grapefruit, though of these the lemon is apparently the best source of the active vitamin.

Citrin, as isolated, forms light yellow crystals sparingly soluble in water but very soluble in alkali, giving intense yellow solutions. These crystals consist mainly of the difficultly water-soluble hesperidin and a small amount of the readily water-soluble eriodictyol. Water solutions of these crystals reverse the situation, containing less hesperidin and more eriodictyol.

Lorenz and Arnold (1939) have prepared a solution from lemon juice suitable for therapeutic study, and Kugelmass (1940) has reported use of such solutions in the treatment of vascular purpuras.

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The probable structure of eriodictyol is shown below:



Eriodictyol or Eriodictin (Vitamin P)

It shows that the vitamin is a flavonol derivative; and Kugelmass suggests that, as it occurs in the natural juice, it might be formularized as sugar-O-R compound in which R represents the flavonol group attached to a carbon of the sugar to form the glucoside. Such a combination (Sugar-O-R) renders the dye group (autochrome group) inactive, so that in the plants the flavonol glucosides are practically colorless. When the sugar is hydrolyzed off, the yellow color develops.

Kugelmass also states that it is known that glucosides in plants are recognized to be capable of immobilizing substances with potential activity until needed in metabolism or detoxification, and that perhaps its value in protecting capillary resistance may reside in its detoxifying power.

Prior to Kugelmass' report, Armentano (1936) had found that paprika juice was actually of value in vascular purpura. Other workers reported conflicting results, Moll (1938) and Zilva (1937) denying its value and King (1939) expressing doubts of its vitamin character. On the other hand, Lajos (1937) reported that in five cases of hemorrhagic nephritis response and healing was obtained by intravenous and oral use of citrin. In 1939 Scarborough reported what he considered positive evidence that for human subjects, citrin

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concentrates contain a factor corrective of capillary fragility and different from ascorbic acid.

Kugelmass (1940) has reported the use of citrin glucosides in four types of vascular purpura. He prepared his dosages by the method of Szent Gyorgyi (1938). The solution he used contained 50 mg. per cc. of flavonones (mixture of hesperidin and eriodictyol) and was given in doses of 150 mg. orally. The types treated were:

- (a) Nutritional purpura
- (b) Allergic purpura
- (c) Infectious purpura
- (d) Mechanical purpura

The treatment proved effective for the first three types but not for the mechanical type.

In connection with these case histories Kugelmass presents the following findings. The citrin did not in any case alter the concentration of any of the blood-clotting factors (fibrinogen, prothrombin, or platelets) in either normal children or in children with hemorrhagic disease. It does not,

Table 15. Effect of Citrin (Vitamin P) on Nutritional and Allergic Purpura.

(After Kugelmass, 1940)

Nutritional Purpura						
Day	5	10	15	20	25	30
Petechiae	51	45	35	30	8	5
Treatment	transfusion		Vitamin C		Vitamin P	

Allergic Purpura						
Day	5	10	15	20	25	30
Petechiae	115	90	48	30	45	22
Treatment	Calcium		Vitamin C		(o)	Vitamin P

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therefore, function like vitamin K. An example of his findings is given in Table 15.

These results indicate that vitamin P needs further consideration and study and may prove a very important factor in treatment of hemorrhagic diseases.

Bibliography

- Armentano, L., Bentsath, A., Beres, T., Rusznyak, Stefan, and Szent Gyorgyi, A., *Deutsch. med. Wochenschr.*, 62, 1325 (1936). Presence of Vitamin P in Paprika.
- Bentsath, A., Rusznyak, Stefan, Szent Gyorgyi, A., *Nature*, 138, 798 (1936). Vitamin P Claim Supported by Effect on Guinea Pigs.
- King, C. G., *J. Amer. Med. Assn.*, 111, 1098 (1938). Physiology of Vitamin C. Vitamin P not a Vitamin.
- Kugelmass, I. N., *J. Amer. Med. Assn.*, 115, 519 (1940). Vitamin P in Vascular Purpura.
- Lajos, C., *Klin. Wochenschr.*, 16, 1615 (1937). Successful Treatment of Hemorrhagic Nephritis with Vitamin P.
- Lorenz, A. J., and Arnold, L. J., Paper Read Before the Amer. Chem. Soc., Sept. 12, 1939. Preparation and Estimation of Crude Citrin Solutions (Vitamin P) from Lemons.
- Moll, T., *Klin. Wochenschr.*, 16, 1653 (1937). Negative Results with Vitamin P.
- Rusznyak, Stefan, *Z. f. Physiol. Chem.*, 249, 214 (1937). The Fate of Parenterally Injected Citrin Solutions in the Animal.
- Rusznyak, Stefan, and Szent Gyorgyi, A., *Nature*, 138, 27 (1936). Vitamin P a Flavone Glucoside.
- Scarborough, H., *Biochem. J.*, 33, 1400 (1939). Definite Effect of Vitamin P on "Capillary Resistance.
- Zilva, S. S., *Biochem. J.*, 31, 915 (1937). Negative Results with Vitamin P.

CHAPTER ELEVEN

THE FUNCTIONS OF VITAMIN D

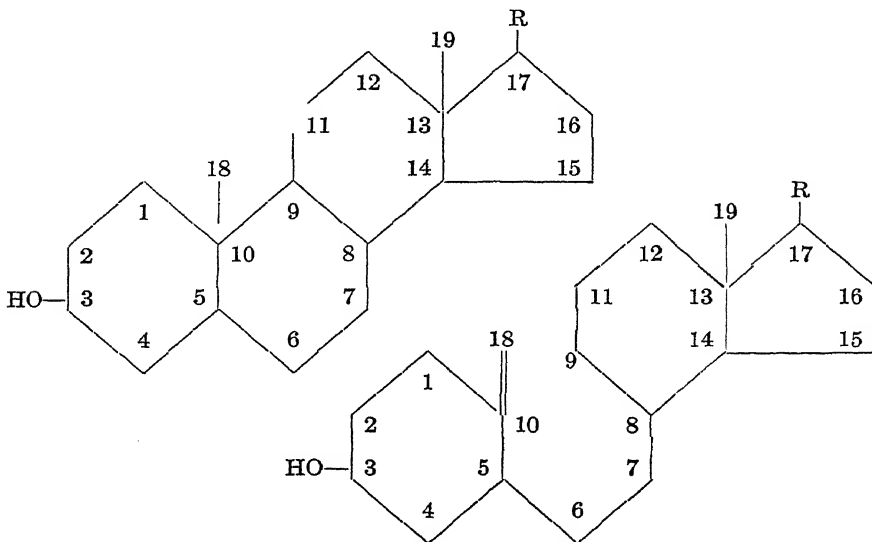
THERE are two forms of vitamin D now available for treatment of rickets; one is what is known as irradiated ergosterol or calciferol, the other is irradiated 7-dehydro-cholesterol. The formulas for these two substances and their comparison with ordinary non-antirachitic cholesterol are shown in the Appendix, Figures 13 and 14. The 7-dehydro-cholesterol form is the one that is present in the human skin and in fish liver oils such as cod liver oil. Calciferol is the type present in viosterols.

These are, however, not the only forms of antirachitic sterols. Bills (1938) has reported that there are at least ten compounds of a sterol type that have rickets-healing potency. With one exception, all of these sterol compounds exist in a non-active or provitamin state and are made active against rickets by irradiation with ultraviolet light. Calciferol is also known as D_2 and 7-dehydro-cholesterol as D_3 . There is no D_1 in the literature today because of the fact that the compound originally given this designation proved to be a mixture of more than one sterol.

Exactly what happens when these sterols are irradiated is still unknown. There is no essential change in the empirical

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formula but it is claimed that irradiation opens a bond between the ninth and tenth sterol positions.



The Sterol Nucleus of Vitamins D Before and After Activation.

For some time it was believed that there was only one vitamin D, namely calciferol. In 1879, Tanret isolated a sterol from ergot to which he gave the name "ergot sterol" or "ergosterol". Ergosterol when irradiated proved to be strongly antirachitic and was the original vitamin D. In 1934 Waddell, studying the treatment of rickets in chicks, found that the vitamin D in cod liver oil was more potent for chicks than calciferol. This led to the search for another form of D and the discovery of 7-dehydro-cholesterol, or vitamin D₃. The term "viosterol" was adopted to indicate a solution of activated ergosterol or calciferol in an inert oil. Ergosterol is the provitamin in yeast; consequently when yeast is irradiated it is calciferol that is produced.

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When ergosterol is irradiated only about fifty per cent of it is actually converted to calciferol, and during the process of irradiation a series of compounds is formed which Bills lists in order of their appearance as follows:

1. Ergosterol
2. Lumisterol
3. Tachysterol
4. Calciferol
5. Toxisterol (Substance 248)
6. Suprasterols I and II.

From such irradiation mixtures lumisterol, the two suprasterols and calciferol have been isolated in the crystalline state. Tachysterol has been separated as a benzoate. Toxisterol has not been isolated in the pure state. It gets the name "Substance 248" because of an absorption band at $248\mu\mu$. Of all these compounds only calciferol has antirachitic action. Lumisterol is convertible into calciferol and also may form with it an additional compound consisting of one part of lumisterol to one part of calciferol. It was this addition product which German workers classed as vitamin D_1 . Toxisterol is not antirachitic and may produce a toxic effect; similarly tachysterol is non-antirachitic and may be slightly toxic. Earlier preparations of irradiated ergosterol sometimes produced toxic effects now believed to be due to failure to eliminate the toxisterols and tachysterols.

As stated above, vitamins D_2 and D_3 appear to be the most abundant forms of antirachitic factor. Bills, however, postulates at least eight others. His list is shown in Table 16.

It has been possible to activate provitamin D in other ways than by bombardment with ultraviolet rays. Knudson (1927)

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Table 16. Antirachitic Sterols.

(After Bills)

A. Structural formulas elucidated:

Calciferol; Vitamin D₂; Activated Ergosterol

7-dehydro-cholesterol; vitamin D₃

22-dehydro-ergosterol; vitamin D₄*

7-dehydro-ergosterol; vitamin D₅

Cholesterilene sulfonate; vitamin D₆

B. Structural formulas not elucidated:

Irradiated 7-hydroxy cholesterol.

Cholesterol freed of normal provitamin and irradiated, but not heated.

Ergosterol heated with nitrites.

Irradiated, heated reaction product of 7-ketocholesteryl acetate and isobutyl magnesium bromide.

Irradiated 22, 23-oxido-ergosterol.

* D₄ may be the significant antirachitic in irradiated cereals.

succeeded in accomplishing activation with cathode rays, and Moore and DeVries (1931) with radium emanations. X-rays and short length radio waves of high intensity were without effect, and ultraviolet irradiation is still the most effective. The wave-lengths which produce this effect range from 230 to 235 $\mu\mu$. It has been found that the solvent is also a factor in determining the efficiency of irradiation, ether solutions of ergosterol providing higher potency solutions than alcohol or cyclohexane solutions. A unit of vitamin D₂ or D₃ is defined as the effect of .000025 mg. of either calciferol or 7-dehydro-cholesterol.

Vitamin D and Rickets

Vitamin D was discovered in the search for a factor preventive of rickets, and to date its principal use in therapeutic

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preparations is as an antirachitic agent. Rickets is a disease of infancy; but further research has shown that the effectiveness of vitamin D is not limited to infancy, and that it is active throughout the life of the individual. Discussion of its function therefore naturally falls into two divisions: First, how does it act in rickets prevention and secondly, how does it behave in later life?

Rickets is defined in medical dictionaries as:

"A constitutional disease of infancy, characterized by impaired nutrition and changes in the bones, the symptoms being a diffuse soreness of the body, slight fever, and profuse sweating about the head and neck, and changes in the osseous system, consisting in a thickening of the epiphyseal cartilages and periosteum and a softening of the bones." (Gould's Med. Dict., 4th Ed., Blakiston, 1936).

By 1921 investigation and speculation in regard to rickets had resulted in two findings of note: first, that cod liver oil was a specific against the disease and, secondly, that exposure to sunlight was also beneficial. In 1921 Mellanby suggested that the vitamin A in cod liver oil might be the significant factor, but at the same time pointed out certain objections to this view. The actual demonstration that the preventive factor was not vitamin A, but a fat-soluble substance in the non-saponifiable part of cod liver oil was made by McCollum in 1922. McCollum named this factor "vitamin D". With this discovery, the relation of sunlight was clarified, because it was shown that human skin contains vitamin D in inactive or provitamin form, and that the impinging of certain ultraviolet rays of sunlight on the skin activates this provitamin, which is then absorbed and utilized by the body.

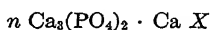
As stated in the preceding paragraphs, the two forms of

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vitamin D most studied and best understood today are known as D₂, or calciferol, and D₃, or 7-dehydro-cholesterol, though at least eight other compounds have been shown to manifest antirachitic effect in greater or lesser degree (see p. 171).

Rickets a Problem of Mineral Metabolism

Chemical analysis of bone shows it to have in general the following composition:



in which n has a value of 2 or 3 and X is mainly CO₃. In other words, bone is mainly calcium phosphate (85%) plus a little calcium carbonate (12%). There may also be present small amounts of magnesium, sodium, potassium and chlorine, but essentially bone is formed by the deposition of calcium and phosphorus in the cartilaginous matrix. The pertinent vitamin D problem then is to find the answer to the question: "How does vitamin D accomplish the proper utilization of the mineral elements calcium and phosphorus?"

Sherman and Pappenheimer in 1921 showed that it is possible to produce rickets in rats by feeding diet 84 shown in Table 17 and to prevent it by feeding diet 85. The

Table 17. Sherman-Pappenheimer Rachitic and Antirachitic Diets.

Ingredients	Rachitogenic	Antirachitogenic
	Diet 84 (%)	Diet 85 (%)
Patent flour	95	95
Calcium lactate	2.9	2.5
NaCl	2.0	2.0
Iron citrate	0.1	0.1
Potassium phosphate	0.0	0.4
Ca/P ratio	6.5/1	3/1

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rachitogenic diet 84 could be made to prevent rickets by the addition of vitamin D. These early experiments of Sherman and Pappenheimer suggested that the cause of rickets was an unsatisfactory balance between calcium and phosphorus in the diet. That principle has been utilized as the basis of the present U.S.P. test for vitamin D potency.

The diet that is used today for producing rickets in this test is not diet 84 but a better balanced diet devised by Steenbock and Black (1925) and known as diet 2965. This diet consists of 76% cornmeal, 20% gluten flour, 3% calcium carbonate and 1% sodium chloride. The calcium content of this diet is about 1.2% and the phosphorus content about 0.25, making the Ca/P ratio between 4 and 5 to 1.

Shohl (1939) has shown that regardless of the ratio, diets become more or less rachitogenic as the absolute amounts of calcium or phosphorus are lowered or raised. In other words, for prevention of rickets the ratio is important; but the absolute amount of intake is equally important, for if the mineral intake is insufficient, retention will not be adequate regardless of whether vitamin D is present or absent. (See also Steenbock *et al.*) Shohl also points out that, since both calcium and phosphorus are necessary for bone formation, any factor which influences the supply or utilization of these elements must be of influence in bone formation. On that basis he would include as rachitogenic factors metals forming insoluble phosphates, such as beryllium, magnesium, strontium, iron, lead and thallium.

Kohman (1939) has also shown that the oxalic acid in spinach, by forming an insoluble calcium oxalate, may not only interfere with the absorption of the calcium from spinach but may even precipitate calcium derived from other

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food substances and cause it to be voided in the feces instead of absorbed.

Shohl (1938) also calls attention to the work of Hamilton and Schwartz (1933) and Hamilton and Dewar (1937) who were able to convert rachitogenic diets into normal diets by the addition of organic acids and alkaline ash, or to produce rachitogenic diets by adding alkalies plus acid ash to normal diets. Hamilton and Schwartz, for instance, used sodium acetate, sodium tartrate, sodium bitartrate, citric acid and tartaric acid. These converted the rachitogenic diets into normal, and the list is given in the order of effectiveness. In the second series they used ammonium carbonate and ammonium chloride. Shohl (1937) believes that the effects with the organic acids were not due simply to the acidity produced, but that the nature of the organic acid itself played a part, and that the citrate ion is definitely more pronounced in effect than the tartrate ion.

Citric acid and alkaline residue added to rachitogenic diets prevented and cured rickets. It is evident, therefore, that there are several ways to control the behavior of ingested calcium and phosphorus other than by Ca/P ratio control. In looking for the action of vitamin D on this control, we must therefore consider more than one way in which it might act. Furthermore, since calcium and phosphorus can reach the bones only by way of the blood supply to that tissue, it becomes necessary to investigate the action of vitamin D on the blood content of these elements.

Swallowed calcium and phosphorus after absorption by the intestine must follow one of three routes: they may be deposited in the tissue, excreted into the gut and rejected with the feces, or excreted with the urine. In clinical rickets

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there is increased excretion of fecal calcium and decreased urinary excretion. Fecal phosphorus excretion is also increased.

Where Does Vitamin D Function?

One of the earliest diagnostic signs of rickets is the change in inorganic phosphate in the blood. This value decreases appreciably in rickets and is restored to normal by vitamin D administration. Calcium content of the blood is also lowered in rickets and raised by vitamin D, but not to the extent of the variations in inorganic phosphate. (See Table 18.)

Table 18. Calcium-Phosphorus Content of Blood.

Clinical Condition	Phosphorus per 100 cc. (mgms.)	Calcium per 100 cc. (mgms.)
Rachitic, no D	3	7
Normal, with D	4.5	10
Hypervitaminosis D	8	15

These changes in blood content would suggest that rickets in some way decreases the absorption into the blood of phosphorus from the digestive tract, and that vitamin D increases such absorption.

Harris (1931) and associates reported that vitamin D increased the net absorption of calcium and phosphorus. Nicollaysen (1939) and associates claimed, however, that vitamin D affects the absorption of calcium, but not that of phosphorus. Recently a new method of observing the fate of phosphorus in the body has been developed. A radioactive isotope of phosphorus (P_{32}) has been separated. By feeding this isotope and then examining with x-ray, it is possible to follow its progress through the body and its

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deposit in specific regions. Using this method Dols *et al.* (1939) have reported that there was no characteristic action of vitamin D on the absorption or re-excretion of phosphorus in the gut of the rachitic rat; also that there was no difference in the rate of phospho-lipid synthesis in the rachitic and non-rachitic rat.

Morgareidge and Manly (1939) have confirmed the viewpoint of Nicollaysen using the same phosphorus isotope; they show that addition of vitamin D does not increase the absorption of phosphorus. Feeding the radioactive phosphorus with Na_2HPO_4 , they showed that the amount appearing in the blood was the same for rachitic and vitamin D rats. On the other hand their experiments indicated that, while the amount of the phosphorus appearing in the bone (metaphysis) was the same for the rachitic and vitamin D rats for the first 54 hours after giving the dose (0.5%), 54 hours later the amount in the metaphysis of the D rats rose 2% higher and that in rachitic rats stayed at 0.5%. These experiments show clearly that the vitamin D actually expedites the delivery of phosphorus to the portion of the bone where it is needed for bone formation, but not by increasing the rate of its absorption from the digestive tract.

Another diagnostic sign of rickets is increase in the enzyme phosphatase in the blood. The enzyme phosphatase, which occurs in various tissues of the body, is able to break down certain organic phosphoric acid combinations or esters by hydrolysis. It has been shown that the cells concerned with bone growth and maintenance contain a high concentration of this enzyme. Kay (1932) has discussed the subject from this viewpoint in detail. The theory is that in the presence of phosphatase organic phosphoric acid compounds are

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broken down to release inorganic phosphate. The concentration of this inorganic phosphate then reaches a point where it combines with the calcium and is precipitated.

Phosphatase is present at all times in the blood serum. The normal level varies in relation to age and rate of growth. It is low at birth, rises to maximum during the first month of life and gradually drops off thereafter, the adult values being about one-fifth of those at the end of the first month. Bodansky and Jaffe (1934) have reported that there is a second rise between the ages of ten and fifteen years, which may be connected with the increase and rate of growth associated with puberty.

Morris and Peden (1937) have discussed the cause of the increase of blood phosphatase in rickets. They draw the general conclusion that this rise indicates the presence of a surplus of unused phosphatase in the bone cells; that in rickets there is a discrepancy between both cell activity and effective supply of calcium and phosphorus, or in the ability of the cells to use these minerals.

The phosphatase rise has attracted attention because apparently it is the earliest sign of rickets, appearing before x-ray or blood phosphate or other clinical signs of rickets are evident. That phosphatase in the serum is a reliable means of detecting the active rachitic states in early and doubtful cases has been confirmed by Barnes and Carpenter (1937) and others. The amount of increase that takes place in rickets has been found to be from two to twenty times the normal value.

There is general agreement that doses of vitamin D should be continued in rickets until the serum phosphatase has returned to normal, but there is some disagreement as to

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Table 19. Blood Phosphatase in Bone Diseases.

(After Kay, 1932)

Condition	Number of Cases	Average Phosphatase Content of Plasma (units)
Normals	33	.14
Osteomyelitis	8	.27
Arthritis with bony changes	7	.17
Myositis ossificans	3	.17
Fragilitas osseum	6	.41
Infantile rickets	13	1.03
Renal rickets	2	1.20
Adolescent rickets	1	2.4 or more
Osteitis fibrosa, generalized	3	1.8
Osteitis fibrosa, focal	7	.21
Osteitis deformans	24	1.7

Table 20. Influence of Vitamin D from Several Sources on the Serum Phosphatase of Chicks.

(After Correll and Wise, 1938)

Groups of Chicks				
Vitamin D per 100 gm. of diet:	None Units	18 I.U. as Cod Liver Oil	37 I.U. as Cod Liver Oil	37 I.U. as Tuna Live
Phosphatase per 100 cc. of serum on				
1st day	71.3	71.3	71.3	71.3
in 2 weeks	158.7	56.4	69.6	81.3
in 4 weeks	267.7	44.1	41.4	65.0
in 6 weeks	248.0	54.8	48.2	115.2
in 8 weeks	240.0	44.0	38.6	76.6

whether healing of rickets is complete when the phosphatase has again reached the normal level if the criterion of healing is the x-ray. It has been suggested, however, that since the x-ray merely shows when the bone has actually been deposited the restoration of the serum to normal phosphatase

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content might naturally precede complete bone deposition; and that such return measures the restoration of the bone-forming ability of the tissue cells to normal.

It has been shown by various workers that in the rachitic condition the rate of basal metabolism is lowered and that the addition of vitamin D tends to restore this rate to normal. Deutsch, Reed and Struck (1936) showed that massive doses of vitamin D increased the basal metabolic rates of normal dogs and rats. Presnall (1937) has shown that the skin of rachitic rats consumed oxygen at a lower rate than that of non-rachitic rats. Reed (1939) concluded from his experiments that the effect of vitamin D on metabolism was not action on the thyroid but on the anterior pituitary, the vitamin probably functioning through the thyreotropic control of the anterior pituitary. Bennholdt-Thompson and Wellman (1934) showed a relation between iodine, thyroid and vitamin D which is summarized in Table 21.

Table 21.

After Bennholdt-Thompson and Wellman (1934)

Iodine Percentage in

Rats	100 cc. Blood	100 mg. Thyroid
Controls	25.2	211
Fed D Concentrate	31.0	167

Dalldorf and Rowe, using *in vitro* iris epithelial cells from the chick and chick embryo juice as the nutrient, showed that they could increase the proliferation of these cells by addition of vitamin D to the medium, but that such increase took place only in the presence of vitamin A.

When we put these various observations together they seem to point to a specific action of vitamin D on cell activity

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with increase of cell metabolism, a retention of phosphatase in the bone and an increased retention of phosphorus itself in the bone-forming region. There is evidence that presence of vitamin D in the gut has some effect on increased absorption of calcium, but not of phosphorus *per se* unless there is a collateral effect associated with control of the hydrogen ion concentration of the gut. A study of the rate of absorption of different forms of phosphorus at different hydrogen ion concentrations was made by Patwardhan, V. N. and Nhavi, N. G. (1939) with the following results:

Orthophosphate was rapidly absorbed at pH 9.4, less rapidly at pH 7.0 and still less rapidly at pH 4.9. Glycero-phosphate was as rapidly absorbed as orthophosphate at pH 7.0 but much more slowly at pH 4.9. Sodium phytate was not absorbed at all at pH 3.8 to 5.2. They suggest that the explanation of these differences is a matter of the extent of hydrolysis of the compound, that the phosphatase which accomplishes the hydrolysis of glycerophosphate in the intestine has optimum effect on the alkaline side, and that consequently the glycerophosphate is not hydrolyzed so rapidly at pH 4.9 as at pH 7.0. If, then, vitamin D should have an effect on the pH of the gut these observations would indicate that it could effect to a degree the actual rise of phosphorus absorption.

There is no question that the effect of vitamin D is enhanced by the presence of adequate amounts of calcium and phosphorus. It has been definitely shown that vitamin D in combination with milk, which is rich in the bone-forming elements, is more effective than vitamin D alone; that in general it requires a lesser number of units when the vitamin D is dissolved in milk than when it is given as cod liver

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oil; and that both cod liver oil and vitamin D milks are more effective in lower unitage than the concentrated viosterol.

In 1921, Howland and Kramer suggested using the product of the blood calcium and blood phosphorus values as an index of the presence or absence of rickets. Under normal conditions this concentration is 10.5 mg. per 100 cc. of calcium and 4.5 mg. per 100 cc. of phosphorus, or a product of approximately 50. Howland and Kramer stated that rickets occurs when this product is less than 40, and that healing commences when the product reaches 40. These data alone, however, were shown not to be applicable to all cases of rickets. They are, however, interesting as showing a relation between bone formation and calcium and phosphorus precipitation.

McLean and Hastings (1935) showed that if the ion product of $\text{Ca} \times \text{PO}_4$ is less than 10^{-27} , calcium phosphate goes into solution; that to inhibit precipitation the product must not exceed $10^{-23.5}$, but that once started, precipitation will continue until an ion product of 10^{-27} is reached. The latter is true only if the proportion of solids to fluid in the solution is greater than 150 mg. per liter.

Other Functions of Vitamin D

Vitamin D has been definitely shown to correct the osteomalacia (morbid softening of the bone) and the hunger osteopathy of adults; during pregnancy it has the ability to prevent loss of calcium from the bones and teeth. MacBeath and Zucker (1938) have produced evidence to show that it is at least one factor in the prevention of dental caries.

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It has also been claimed that vitamin D aids in fighting infection, but at the present time there is little to support this view. It may be that in favoring calcification, vitamin D might be of some aid in sealing off of tuberculous foci; but here again the action of the vitamin is not specific and it produces no anti-bodies or anti-toxins.

Vitamin D and Skin Lesions

We have already noted that vitamin D (Dalldorf and Rowe) can affect the rate of proliferation of epithelial cells and that it also (Presnall) affects respiration or oxygen uptake of the skin. Doktorský and Platt (1933) reported reduction in pustule count in acne by treatment with 5000-6000 units of vitamin D daily. Of thirty-five cases, thirty-one showed 70-80% improvement. Hinrichsen and Ivy (1938), basing their opinions on the treatment of 210 cases of acne with two levels of dosage (20,000-30,000 units), reached the conclusion that while vitamin D is not a specific remedy for acne it is a valuable agent. Comel (1935) found vitamin D beneficial in certain eczema cases and believes the effect is due to improvement of cell metabolism. Maynard (1928) got better effect on acne with vitamin D than with x-ray, and Cornbleet and Schick (1937) found high dosage (200,000-300,000 units) beneficial in scleroderma. McLaughlin (1939) has reported definite improvement in the healing of x-ray burns by adding vitamin D to the healing lotion. Ceder and Zon (1937) have presented convincing evidence of benefit in cases of psoriasis by large doses of vitamin D, though not all their cases responded.

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“Pollinosis”, or hay fever and asthma, have been treated with massive doses of vitamin D; and Rappaport and associates (1933, 1934) report some synergy between the vitamin and pollen desensitization. The action may be related to calcium control, as beneficial effects were associated with hypercalcemia.

The fact that vitamin D can be absorbed by and through the skin has been abundantly established. In fact, the production of vitamin D through sunlight exposure to ultraviolet rays is explained on the theory that the vitamin exists in the skin in a provitamin condition; the sun rays activate this and the active vitamin D then passes through the skin and into the blood and thence to the bones. Like vitamin A, it has been held to be of definite value in stimulating wound healing.

Blackberg and Knapp (1937) found vitamin D dosage beneficial in treating a distention of the cornea of the eye known as keratoconus, and it has been tried empirically in the treatment of a considerable variety of clinical problems, for example, for cutaneous ulcers, for encysting trichinae, and for combatting lead and radium poisoning. There is little to report on these studies, though evidence of some value has been given.

Arthritis

Reed and associates (1939) have studied the effect of high dosage of vitamin D on arthritis. The response has been positive in some cases but not universally. We cannot at present, therefore, feel sure that vitamin D has any specific relation to the arthritic condition.

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The Relation of Vitamin D to Ultraviolet Light

The provitamins D all respond to certain wave-lengths in the ultraviolet region to become active vitamin D. Knudson and Benford (1938) have reported a relation between the ultraviolet wave-lengths and efficiency in healing rickets and in forming vitamin D (see Table 22). It is of interest

Table 22.

Wave-length (Angstrom)	Energy to Produce ++ Healing* (ergs)	Energy to Form One I.U. of Vitamin D (ergs)	Efficiency Compared with Wave-length 2804 (per cent)
2653	948,600	287,000	79
2804	774,000	226,000	100
2894	1,305,000	395,000	57
2967	927,000	280,000	81
3024	1,976,000	353,000	39
3128	91,000,000	27,545,000	1

* On the basis of findings in the authors' laboratory, a + + healing was considered equivalent to 3.3 International Units.

in studying this table to note that the maximum efficiency was obtained by wave-length 2804 Angstrom. The authors point out that the most effective wave-length, 2804, is shorter than any which reach the earth from the sun even in summer. Wave-length 2967, which is 81 % as efficient as 2804, reaches the earth in the summer in the north temperate zone, if the atmosphere is free from fog, dirt, smoke and clouds. In winter, the shortest wave-length reaching the earth is 3049; hence, at this time, the antirachitic efficiency of solar ultraviolet is even less than that of wave-length 3024, which is shown in Table 22 to be only 39% as efficient as 2804. The authors bring out that this finding explains the marked

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increase of rickets in winter months and decrease of effectiveness of sunshine in those same months.

In connection with ultraviolet radiation, it should be borne in mind that too much irradiation is as bad as too little. Over-exposure may destroy the vitamin D that has been formed.

One other thing that should be remembered regarding the use of ultraviolet to stimulate vitamin production is that these waves can penetrate only a short distance into the skin. Consequently, if the skin is covered even lightly by clothing the sunlight will be inactive. Sunshine filters such as are now used to reduce danger of sunburn should not be 100% effective. If the ultraviolet is completely screened out, the health benefits are lost. It has been demonstrated that ordinary window glass cuts out the activating ultraviolet rays; hence, sunlight received through it has no antirachitic effect. There are, however, glasses containing certain chemicals which make them able to transmit up to about 60% of the available ultraviolet, and clear fused quartz gives 100% transmission.

Vitamin D Requirements

With adequate supplies of calcium and phosphorus, it has been shown that infants have been protected against rickets by daily dosages as low as 120-130 units, but it is generally recommended that during the period of infancy the daily preventive dose should be in the neighborhood of 400 units. If vitamin D milks are used for this purpose (and they are very satisfactory because they provide at the same time the calcium and phosphorus necessary), it should be borne in

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mind that irradiated milks provide about 135 units per quart, and fortified and metabolized milks run 400-435 units per quart. Ordinary cow's milk is higher in vitamin D content in summer than in winter, but the range is only about 4 or 5 units per quart, which may increase to 40 during the summer months. The amount required during childhood and adolescence is not sharply determined nor is the requirement of adults, but Jeans (1938) has suggested that 300-400 units per day is probably desirable throughout life. In pregnancy and lactation, when there is a sudden increased demand for vitamin D, the minimum requirement has been put at 800 units.

There has been a great deal of controversy in past years over the relative merits of different sources of vitamin D, especially with discovery that chicks responded differently from rats to the type of vitamin D used. The atmosphere is now considerably cleared with the proof that the human being and the rat are apparently equally responsive to both D_2 and D_3 . What vitamin D source to use, then, depends more on the dispersion and absorbability of the vitamin in the medium than on the kind of vitamin D present. Rat unit for rat unit, the value of the different sources is practically equivalent so far as human treatment is concerned.

There has also been some discussion of method of treatment—whether to use daily doses or whether it is possible to give a large dose and have it carry over on succeeding days. Harnapp (1938) developed a method known as the vitamin D-STOSS therapy. By this method he gave 200,000 to 400,000 units in one dose and claimed that such a dose would protect infants and children throughout the winter or cure relatively severe cases of active rickets.

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Reed states that his daily oral administration of 20,000 units per kilogram of body weight is the upper limit, and that there is less danger of toxicity if the doses are divided. For example, 8000 units per kilogram per day may cause toxic symptoms in an individual in 10 days, although the same individual tolerated 4000 units per kilogram per day for 30 days or longer without toxic symptoms. The advantages of the STOSS therapy need further evaluation.

Bibliography

- Barnes, D. L., and Carpenter, M. D., *J. Pediatr.*, 10, 596 (1937). Comparative Study in Diagnosis and Treatment of Rickets.
- Bennholdt-Thompson, C., and Wellman, M., *Klin. Wochenschr.*, 13, 800 (1934). Relation Between Ultraviolet Light, Iodine, Thyroid, and Vitamin D.
- Bills, C. E., *J. Amer. Med. Assn.*, 110, 2150 (1938). Chemistry of Vitamin D.
- Blackberg, S. N., and Knapp, A. A., *Am. J. Opth.*, 20, 405 (1937). Influence of Vitamin D-Calcium Phosphorus Complex in Production of Ocular Pathology.
- Bodansky, A., and Jaffe, H. L., *Arch. Int. Med.*, 54, 88 (1934). Phosphatase Studies: Serum Phosphate and Diseases of the Bone.
- Ceder, E. T., and Zon, L., *U.S.P.H. Report*, 52, 1580 (1937). Treatment of Psoriasis with Massive Doses of Crystalline D and Irradiated Ergosterol.
- Comel, M., "Yearbook Dermat. and Syph.," pp. 446 and 534, 1935. Vitamin D in Eczema.
- Cornbleet, T., and Schick, H. C., *Arch. Dermat. and Syph.*, 35, 188 (1937). Calcium Metabolism in Scleroderma.
- Correll, J., and Wise, E. C., *J. Biol. Chem.*, 126, 573 (1938). Phosphatase Determinations in Chicks.
- Dalldorf, G., and Rowe, E., Unpublished data, 1937. Vitamin D and *in vitro* Growth Stimulation of Iris Epithelium.
- Deutsch, H., Reed, C. I., and Struck, H. C., *Am. J. Physiol.*, 117, 1 (1936). The Role of the Thyroid in the Calorigenic Action of Vitamin D.
- Doktorsky, A., and Platt, S. S., *J. Amer. Med. Assn.*, 101, 275 (1933). Vitamin D in the Treatment of Acne Vulgaris.

VITAMIN D

- Dols, M. J. L., Jansen, B. C. P., Sizoo, G. J., and van der Maas, G. J., *Proc. Koninkl Akad. Wetenschappen Amsterdam*, 17, 1 (1939). Use of Radio-Active Phosphorus to Show Greater Intensity of Phosphorus Deposit in Vitamin D Supplied Rats.
- Hamilton, B., and Dewar, M. M., *Am. J. Dis. Chil.*, 54, 548 (1937). Effect of Citrate and Tartrate in Experimental Rickets.
- Hamilton, B., and Schwartz, C., *Am. J. Dis. Chil.*, 46, 775 (1933). Rickets and Hyperthyroidism.
- Harnapp, G. O., *Klin. Wochenschr.*, 17, 390 (1938). Die Stross Prophylaxie der Rachitis mit Vitamins D₂ and D₃.
- Harris, L. J., *Lancet*, 218, 236 (1930); 222, 1031 (1932). The Mode of Action of Vitamin D and Relation to Calcium and Phosphorus Intake.
- Harris, L. J., and Innes, J. M. R., *Biochem. J.*, 25, 367 (1931). Mode of Action of Vitamin D.
- Hinrichsen, J., and Ivy, A. C., *Illinois Med. J.*, 74, 85 (1938). The Value of Irradiated Ergosterol in the Treatment of Acne Vulgaris.
- Howland, J., and Kramer, B., *Am. J. Dis. Chil.*, 22, 105 (1921). Calcium and Phosphorus in Relation to Rickets.
- Jeans, P. C., and Stearns, G., *J. Amer. Med. Assn.*, 111, 703 (1938). The Human Requirement of Vitamin D.
- Kay, H. D., *Physiol. Rev.*, 12, 384 (1932). Phosphatase in Growth and Disease of Bone.
- Knudson, A., *Science*, 66, 176 (1927). Further Studies on the Antirachitic Activation of Substances by Cathode Rays.
- Knudson, A., and Moore, C. W., *J. Biol. Chem.*, 81, 49 (1929). Comparison of Antirachitic Potency of Ergosterol Irradiated by Ultraviolet Light and by Exposure to Cathode Rays.
- Kohman, E. F., *J. Nutr.*, 18, 233 (1939). Oxalic Acid in Foods and Its Behavior and Fate in the Diet.
- MacBeath, E. C., and Zucker, T. F., *J. Nutr.*, 15, 547 (1937). The Role of Vitamin D in the Control of Dental Caries in Children.
- McCollum, E. V., *et al.*, *J. Biol. Chem.*, 45, 333 (1921); 51, 42 (1922). Studies in Experimental Rickets.
- McLaughlin, R. R., *Medical Times*, July, 1939. Skin Healing and Vitamin D.
- McLean, F. C., and Hastings, A. B., *Tr. A. Am. Physicians*, 49, 76 (1934). *J. Biol. Chem.*, 107, 337 (1934), 108, 285 (1935). *Am. J. Med. Sci.*, 189, 601 (1935). Calcium Ion Concentration in Blood and Body Fluids.

WHAT ARE THE VITAMINS?

- Maynard, M. T. R., *Calif. and West. Med.*, 49, 127 (1938). Vitamin D in Acne.
- Mellanby, E., Spec. Report Series No. 61, Med. Res. Council, 1921. Experimental Rickets.
- Moore, R. B., and DeVries, T., *J. Am. Chem. Soc.*, 53, 2676 (1931). The Activation of Ergosterol with Radium Emanation.
- Morgareidge, K., and Manley, M. L., *J. Nutr.*, 18, 411 (1939). Simultaneous Appearance of a Positive Line Test and Radioactive Phosphate Deposition in the Rachitic Rat Metaphysis.
- Morris, N., and Peden, O. D., *Quart. J. Med.*, 6, 211 (1937). Plasma Phosphatase in Disease.
- Nicollaysen, R., and Jansen, J., *Arch. Pediatr.*, 23, 405 (1939). Vitamin D and Bone Formation in Rats.
- O'Brien, B., and Morgareidge, K., *J. Nutr.*, 18, 1939; 16, 395 (1938). Effect of Phosphorus on the Biological Estimation of Vitamin D Activity.
- Patwardhan, V. N., Nhavi, N. G., *Biochem. J.*, 33, 663, 1939. Relation of hydrogen ion to phosphate absorption.
- Presnall, K., *J. Biol. Chem.*, 121, 5 (1937). The Relation of Vitamin D to Skin Respiration.
- Rappaport, B. Z., Reed, C. I., Hathaway, M. L., and Struck, H. C., *J. Allergy*, 5, 541 (1934). The Treatment of Hay Fevers and Asthma with Viosterol of High Potency.
- Reed, C. I., Struck, H. C., and Steck, I. E., "Vitamin D" (text). Univ. Chicago Press, 1939.
- Sherman, H. C., and Pappenheimer, A. M., *J. Exper. Med.*, 34, 189 (1921). A Diet Producing Rickets in White Rats and Its Prevention by Addition of an Inorganic Salt.
- Shohl, A. T., *J. Nutr.*, 14, 69 (1937). The Effect of Acid-Base Content of the Diet Upon the Production of Rickets with Special Reference to Citrate. *J. Amer. Med. Assn.*, 111, 614 (1938). Physiology and Pathology of Vitamin D. "Mineral Metabolism," Reinhold Publishing Corp., 1939.
- Shohl, A. T., and Wolbach, S. B., *J. Nutr.*, 11, 275 (1936). Rickets in Rats.
- Steenbock, H., and Black, A., *J. Biol. Chem.*, 64, 263 (1925). Fat-Soluble Vitamins XXIII: Diet 2965.
- Tanret, M. C., *Ann. chim. phys.*, Series 5, 17, 493 (1879). De l'ergotinine.
- Waddell, J., *J. Biol. Chem.*, 105, 711 (1934). The Provitamin of Cholesterol.

CHAPTER TWELVE

THE FUNCTIONS OF VITAMIN E

IN 1936 Evans and the Emersons (1936) reported the extraction of an oily substance which exhibited vitamin E activity in doses of one milligram. A single dose of 3 mg. permitted the regular production of normal litters of rats under nutritive conditions not ordinarily favoring normal gestation. To this substance the investigators gave the name of alpha-tocopherol. The name is derived from "tokos" meaning "childbirth", and "phero" meaning "to bear", the ending "ol" indicating an alcohol.

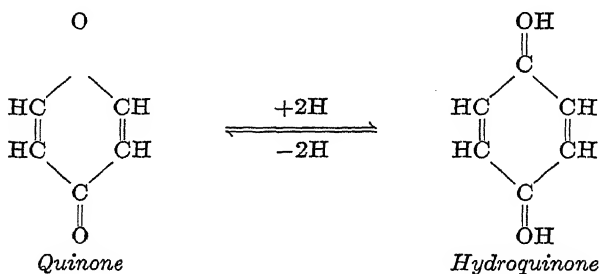
Two crystalline derivatives of this active oil were prepared and an elementary formula of $C_{29}H_{50}O_2$ was derived for the vitamin itself. Two additional alcohols were also obtained as oils from the wheat germ concentrate, each of which proved to be isomeric with alpha-tocopherol. One of these was physiologically inactive, but the other appeared to have some vitamin E potency. The structure of these tocopherols is given in the Appendix.

Vitamin E was first postulated by Evans and Bishop (1922) and the summary of this earlier work has been given by Evans (1932). They discovered that rats, when reared on

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diets otherwise complete but lacking a fat-soluble factor, did not have offspring, though they were apparently normal in other respects. The females failed to carry their young to term; embryos died and were resorbed, but the female reproductive mechanisms were not damaged since adequate doses of the vitamin restored fertility. Male animals deprived of this factor, however, became sterile through degeneration of the germinal epithelium, and vitamin E dosage was ineffective in restoring them. It was also demonstrated that the substance could be destroyed by oxidation. Mattill (1938) has reviewed later phases of the vitamin E chemistry.

As shown in the formula this vitamin contains the quinone nucleus which is well known to act as a hydrogen acceptor:



In 1934 Olcott and Mattill obtained a concentrate of vitamin E from wheat germ oil which was found to have antioxidant action as well as the physiological effect described by Evans and Bishop. This anti-oxidizing effect was destroyed by acetylating the compound, but this acetylation did not destroy the biologic activity. This discovery, and further proof by Olcott (1935) that the vitamin contained a hydroxyl group which was easily esterified, was one of

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the steps that led to the elucidation of the structural character of vitamin E, or alpha-tocopherol (see p. 214).

The role of vitamin E in the reproduction of rats has been fully established. It is essential, as stated above, for the production of normal litters by the female and to prevent sterility in the male. We have very little data to determine whether these results with rats are directly transferable to other animals. Bay and Vogt-Moller (1934) found that intramuscular or preferably subcutaneous injection of a sterilized wheat germ oil into cows repeatedly failing to become pregnant was followed by pregnancy in 33 out of 50 instances. These results were confirmed by Tutt. Schioppa (1935) claimed that large doses of wheat germ oil increased the size of rabbit litters, and several authors have reported that the hatchability of hens' eggs depends to a degree on the vitamin E content of the egg and the vitamin E diet of the hen (Barnum, 1935). Adamstone (1934), from studies of the effect of vitamin E on male fowl, reported that it is probably intimately associated with the behavior of the nucleus during cell division. The striking difference in response to vitamin E deficiency in male and female animals would appear to be that in the male the damage is done to part of the animal's own tissue, whereas in the female the damage is to the fetus and not to the female's own tissue.

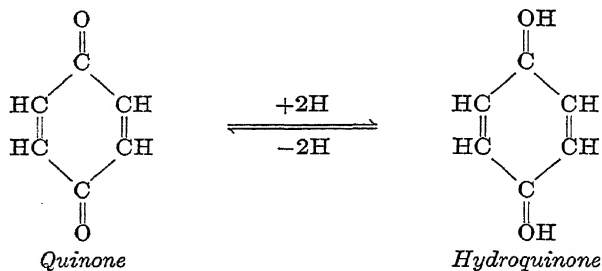
Mason (1933) also agrees that vitamin E probably plays some essential role in nuclear activities involving chromatin, and is apparently indispensable especially in tissues in which cellular reproduction and differentiation are rapid.

Blumburg (1935) fed young rats for a period of time on a vitamin E-deficient diet. Retardation of growth occurred at the twelfth to fourteenth week and complete cessation of

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growth in eighteen to twenty-two weeks. He also noted serious malnutrition and some muscular disturbance at thirty to forty weeks. Addition to the diet of vitamin E in the form of wheat germ or the non-saponifiable portion of the oil produced a resumption of growth. Ringsted (1935) also noted this muscular change and dragging of the hind limbs at from five to six months of age.

In 1931 Goettsch and Pappenheimer described a form of muscular dystrophy in guinea pigs and rabbits on a diet deficient in vitamin E, and in 1939 Goettsch and Ritzman reported that alpha-tocopherol prevented the development of muscular dystrophy in young rats when fed from the tenth to the twenty-fifth day after birth. Mackenzie and McCollum (1939) showed that alpha-tocopherol would prevent muscular dystrophy in rabbits placed on the vitamin E-free diet of Goettsch and Pappenheimer. Morris (1939) has confirmed the experiments of Mackenzie and McCollum and has found that individual doses of 20 mg. were close to the requirement of alpha-tocopherol for the cure of muscular dystrophy in rabbits. Whether a similar condition in human beings is remediable in this factor is still unknown.

In human experimentation previous to the discovery of its possible relation to muscular dystrophy, the principal interest has been in this vitamin's prevention of abortion. Various investigators have reported success in the use of vitamin E in such prevention (Watson 1936, Currie 1937, Vogt-Moller and Cromer 1938).

Verzar (1929) claims that vitamin E acts like an anterior hypophysis hormone. This has been disputed, but Van Wagenen (1935) and Stone (1940) state that changes do take place in the anterior hypophysis in E-deficient animals.

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These suggestions need further investigation for confirmation.

At the present time there does not exist sufficient evidence to indicate a general deficiency of vitamin E in the American diet as it appears to be quite widely distributed in natural foodstuffs. For that reason, the Council of Pharmacy of the American Medical Association declined to allow claims of benefit from pharmaceutical preparations of this product. Owing to its antioxidant effect, wheat germ oil is being quite extensively used today in vitamin A-containing capsules to protect vitamin A against oxidative destruction. The isolation and synthesis of alpha-tocopherol now makes possible the studies of the behavior of the pure compound and should lead to a better understanding of the functions of this particular vitamin.

One of the reasons for doubt as to the adequacy of diets in this factor has been the meagerness of data as to distribution in natural foods. Chemical methods of assay and availability of the synthetic product promise remedy of this deficiency of present knowledge.

Bibliography

- Barnum, G. L., *J. Nutr.*, 9, 621 (1935). The Vitamin E Content of Eggs as Related to the Diet of the Hen and Hatchability.
- Bay, F., Vogt-Moller, P., *Vet. J.*, 90, 288 (1934). Treatment of Sterility in Cows and Breeding Sows with Wheat Germ Oil.
- Blumberg, H., *J. Biol. Chem.*, 108, 227 (1935). A Growth Deficiency Disease Curative by Wheat Germ Oil.
- Cromer, J. K., *Med. Annals, District of Columbia*, 7, 137 (1938). Wheat Germ Oil in Treatment of Spontaneous and Threatened Abortion.
- Currie, D. W., *Brit. Med. J.*, 1, 752 (1936). Vitamins for Habitual Abortion.
- Evans, H. M., *J. Amer. Med. Assn.*, 99, 469 (1932). Vitamin E.

WHAT ARE THE VITAMINS?

- Evans, H. M., and Bishop, K. S., *Am. J. Physiol.*, 63, 396 (1922). On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction.
- Evans, H. M., Emersons, O. H., and G. A., *J. Biol. Chem.*, 113, 319 (1936). The Isolation from Wheat Germ Oil of an Alcohol, Alpha-tocopherol, Having the Properties of Vitamin E.
- Goettsch, M., and Pappenheimer, A. M., *J. Exper. Med.*, 54, 145 (1931). Nutritional Muscular Dystrophy in the Guinea Pig and Rabbit.
- Goettsch, M., and Ritzman, J., *J. Nutr.*, 17, 371 (1939). Protection Against Muscular Dystrophy with Alpha-tocopherol.
- Mackenzie, C. G., and McCollum, E. V., *Science*, No. 2312 (1939); *J. Nutrition*, 19, 345 (1940). Alpha-tocopherol Curative of Muscular Dystrophy in Rats.
- Mason, K. E., *Ann. J. Anat.*, 52, 153 (1933). Diff. in Testis Injury and Repair After Vitamin A Deficiency, Vitamin E Deficiency, and Inanition.
- Mattill, H. A., *J. Amer. Med. Assn.*, 110, 1831 (1938). Vitamin E.
- Morris, S. G., *Science*, 90, 424 (1939). Synthetic Alpha-tocopherol and Nutritional Muscular Dystrophy.
- Olcott, H. S., *J. Biol. Chem.*, 110, 695 (1935). Evidence for the Presence of a Hydroxyl Group in Vitamin E.
- Olcott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 104, 423 (1934). Some Chemical and Physical Properties of Vitamin E.
- Ringsted, A., *Biochem. J.*, 29, 788 (1935). Paresis in Adult Rats Suffering from Chronic Avitaminosis E.
- Schioppa, L., *Z. f. Vitaminsforsch.*, 5, 22 (1936). Influence of Vitamin E on Fertility and Bodily Condition of Offspring.
- Shute, E., *Am. J. Obstet. and Gynecol.*, 35, 810 (1938). Wheat Germ Oil Therapy and Effect on Congenital Anomalies.
- Stone, S., *J. Amer. Med. Assn.*, 114, 2187 (1940). Treatment of Muscular Dystrophy and Allied Conditions with Vitamin E.
- Tutt, J. F. Quoted by Bay and Vogt-Moller.
- Van Wagenen, G., *Anat. Rec.*, 29, 398 (1925). Histological Changes in the Male Rat Hypophysis Following Degeneration of the Germinal Epithelium.
- Verzar, F., *Mayo Clinic Proc.*, 4, 351 (1929). The Influence of Diet on Internal Secretion.
- Vogt-Moller, P., *Acta Path. et microbiol. Skand.*, 12, 115 (1935). Estimation of the Vitamin E Content of Fertilan.
- Watson, E. M., *Canad. Med. Assn. J.*, 34, 125 (1936). Clinical Experiences with Wheat Germ Oil and Abortions.

CHAPTER THIRTEEN

THE FUNCTIONS OF VITAMIN K

THE first report to recognize the existence of vitamin K was that of Dam of Copenhagen (1935), though McFarlane and associates (1931) noted that chicks fed on ether-extracted fish meal showed 50% of deaths due to bleeding. Dam noted this same bleeding phenomenon, and also that it could be controlled by administration of the non-saponifiable, non-sterol fraction of hog liver fat, or by feeding alfalfa. Because the unknown factor controlled prothrombin fall in blood and aided coagulation, Dam called it the "Koagulation vitamin" or vitamin K.

The development of the chemistry of this factor has been exceedingly rapid. Two natural forms, K_1 and K_2 , have been identified and described, and the product has been synthesized. Its relation has also been shown to the phthiocol isolated by Anderson and Newman (1933). For details on structure see p. 215.

Prothrombin and Blood Coagulation

There are various theories of blood coagulation, but in general it is agreed that the clot is formed by the conversion

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of fibrinogen to fibrin by a ferment called thrombin. Thrombin does not exist ready-formed in circulating blood, but in a prothrombin state. This prothrombin is converted into thrombin by combination with ionized calcium and by the action of a phospho-lipid of the blood platelets called cephalin or thromboplastein. Blood clotting therefore proceeds in two steps:

- (1) Prothrombin + thromboplastein + calcium = thrombin.
- (2) Fibrinogen + thrombin = fibrin.

On the assumption that blood-clotting rate is proportional to the concentration of thrombin, Quick (1938) developed a method of making clotting time an actual measure of the prothrombin content of blood. As the effect of vitamin K is to increase this content, Quick's test is now in general use clinically to estimate the effect of vitamin K preparations on the prothrombin content of human blood. Prothrombin is believed to be formed in the liver. It is therefore obvious that if vitamin K is deficient in amount, or if it is not absorbed into the portal vein blood from the gut, prothrombin production falls, blood-clotting ability is lowered, and hemorrhage may result.

The satisfactory absorption of vitamin K from the gut requires the presence of bile or bile salts.

Methods have been devised for estimating the distribution of vitamin K in foodstuffs (Almquist and Stokstad, 1938; Ansbacher, 1940) but have not been long enough in operation to permit tabulation of distribution in common foodstuffs. To date it appears to be abundant in parts of plants which show active photosynthesis. Alfalfa, spinach, kale,

VITAMIN K

dried carrot tops, chestnut leaves, tomatoes, and oat sprouts contain it in significant amounts. Certain bacteria or other micro-organisms appear to be capable of synthesizing it, and it is extractable from putrefied fish-liver meal, rice bran and casein. Almquist and associates claim to have identified a specific K-producing organism from fish meal similar to *B. cereus*. The ability, however, appears to be shared by other forms, since K has been extracted from *Eschericia coli*, *B. subtilis*, and *staphylococcus aureus*. This fact means that under certain conditions it may be possible for the vitamin to be synthesized in the human gut when no dietary source is fed.

It is not yet known whether vitamin K enters into the formation of prothrombin as a chemical constituent or merely keeps certain tissues in a state of activity essential to prothrombin formation. It is known that in the absence of bile salts, fed vitamin K is not readily absorbed from the gut. It has long been known that bile salts aid the absorption of fats from the gut, and vitamin K has the solubility properties of fats.

Butt, Snell, and Osterberg (1938) state:

"Numerous other investigators have demonstrated definitely that cholemic bleeding is caused by deficiency of prothrombin in the circulating blood and that both this deficiency and the hemorrhagic state associated with it can be corrected by the administration of concentrates containing the fat-soluble anti-hemorrhagic vitamin K, together with bile salts to insure absorption of the vitamin. The early clinical application of this knowledge concerning vitamin K was begun independently in the United States by Warner and his associates (1938) at the University of Iowa and by us, and abroad by Dam and his co-worker in Copenhagen.

"The earliest reported case of fatal bleeding in a patient having jaundice was made by Wedelius in 1683. Since the advent of Lis-

WHAT ARE THE VITAMINS?

terian surgical technic, the tendency to bleed which is peculiar to patients having jaundice has been a factor of grave concern to the surgeon. In the early reports by Musser and Keen (1884), DePage (1889), Smith (1891) and Robson (1904), hemorrhage accounted for a large part of the high mortality that accompanied the surgical treatment of the jaundiced patient. Even current figures indicate that cholemic bleeding has accounted for about 50 per cent of the mortality accompanying surgical intervention on patients having jaundice and that cholemic bleeding, of itself, imposes a surgical risk of approximately 5 per cent.

"There is now general agreement that hemorrhagic diathesis in the presence of jaundice is not the result of any alteration in the amounts present of calcium, bilirubin, platelets, fibrinogen or thromboplastin. The original suggestion of Quick and his co-workers (1935) that the condition depended on a lack of the one substance necessary for coagulation not previously studied, namely prothrombin, has now been amply confirmed. Evidence has also accumulated to prove that a particular fat-soluble material (that is, vitamin K) normally present in the intestinal tract, is absorbed and utilized by the liver in some unknown manner to maintain a normal concentration of prothrombin in the blood plasma."

With increasing availability of vitamin K in pure form clinical study of K deficiency is making rapid progress. At first its effect in obstructive jaundice received greatest attention, but the scope of the vitamin has widened considerably as the studies have progressed. Waddell and Guerry (1939) called attention to its potential value in saving infant lives in the first weeks. Brinkhous and associates (1937) noted that the prothrombin level in normal new-born babies is only 14 to 39% of that found in the normal adult. Waddell suggests that vitamin K administered immediately after birth may serve to check bleeding and render harmless a slow oozing hemorrhage which might otherwise well cause death or permanent mental and physical crippling. In cases of sprue

VITAMIN K

and ileitis, poor absorption of K may make its administration in greater amounts or by other routes important.

It is still too early to attempt a comprehensive review of clinical states associated with K deficiency, but wherever bleeding occurs the possibility of K deficiency is worth checking, provided that blood tests show low prothrombin content.

Bibliography

- Adamstone, F. B., *J. Morphol. and Physiol.*, **52**, 47 (1931); *Science*, **80**, 450 (1934). Relation of Vitamin E to Blastoderm Structure and Nuclear Development.
- Almquist, H. J., *J. Biol. Chem.*, **120**, 635 (1937). Method of Concentrating K from Alfalfa.
- Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, **61**, 1610, 1611 (1939). Producing by Bacteria. Reported Phthiocol Active.
- Almquist, H. J., Penhater, C. F., and Mecchi, E., *Proc. Soc. Exper. B. & M.*, **38**, 336 (1938). Producing by Bacteria.
- Almquist, H. J., and Stokstad, E. L. R., *J. Biol. Chem.*, **111**, 105 (1935). Fish-meal Putrefied Yielded Vitamin K.
- Anderson, R. J., and Newman, M. S., *J. Biol. Chem.*, **101**, 773 (1933); **103**, 197 (1933). Identification of Phthiocol.
- Ansbacher, S., *Proc. Soc. Exper. B. & M.*, **44**, No. 2 (1940). New Type of Vitamin K-Deficient Diet. See also: Fernholz *et al.*, *J. Am. Chem. Soc.*, **62**, Nos. 2 and 6 (1940).
- Ansbacher, S., and Fernholz, E., *J. Am. Chem. Soc.*, **61**, 1924 (1939). Simple Compounds with Vitamin K Activity. *J. Am. Chem. Soc.*, **61**, 1932 (1939). Vitamin K Value of 2-Methyl-1, 4-Naphthoquinone. *J. Am. Chem. Soc.*, **61**, 1924 (1939); *Science*, **90**, 215 (1939). Activity of Phthiocol.
- Ansbacher, S., Fernholz, E., and Doliver, M. A., *J. Am. Chem. Soc.*, **62**, 155 (1940); *Proc. Soc. Exper. B. & M.*, **43**, No. 4 (1940). Forms of 2-Methyl-1, 4-naphthohydroquinone.
- Binkley, S. B., McCorquodale, D. W., Cheney, S. A., Thayer, S. A., McKee, R. W., and Doisy, E. A., *J. Am. Chem. Soc.*, **61**, 1612 (1939). Isolation of K₁ and K₂.

WHAT ARE THE VITAMINS?

- Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meeting Mayo Clinic*, 13, 753 (1938). Effect on Jaundice.
- Dam, H., *Biochem. Z.*, 215, 475 (1929); 220, 158 (1930). Chick Abnormalities in Sterol Metabolism. *Biochem. J.*, 29, 1273 (1935). Announcement of Vitamin K. *Ann. Rev. Biochemistry*, 1940. Fat-soluble Vitamins.
- Dam, H., Glavind, J., and Svendsen, I., *Biochem. J.*, 32, 485 (1938). Dam Unit.
- Dam, H., and Schonheyder, F., *Biochem. J.*, 30, 897 (1936). Dam's Standard Reference Material Containing 500 Dam Units per Gram (Dried Spinach). *Biochem. J.*, 32, 485 (1938). Method.
- Doisy, E. A., Binkley, S. B., Thayer, S. A., and McKee, R. W., *Science*, 91, 58 (1940). Review of Vitamin K Development.
- Fieser, L. R., Bowen, D. M., Campbell, W. P., Fry, E. M., and Gates, M. D., Jr., *J. Am. Chem. Soc.*, 61, 1926 (1939). Synthesis of Vitamin K.
- Greaves, J. D., and Schmidt, C. L. A., *Proc. Soc. Exper. B. & M.*, 37, 43 (1937). Need for Bile Salts in Absorption from Vitamin K.
- McFarlane, W. D., Graham, W. R., Jr., and Richardson, F., *Biochem. J.*, 25, 358 (1931). The Fat-soluble Vitamin Requirements of the Chick. 1. The Vitamin A and Vitamin D Content of Fishmeal and Meatmeal.
- McKee, R. W., Binkley, S. B., McCorquodale, D. W., Thayer, S. A., and Doisy, E. A., *J. Amer. Chem. Soc.*, 61, 1295 (1939). Isolation of K₁ and K₂. K₁ from Alfalfa Has Light Yellow Oil Crystallizing from Acetone or Alcohol. K₂ from Putrefied Sardine Meal, Light Yellow, Crystalline, Solid.
- Osterberg, A. E., *Proc. Staff Meeting Mayo Clinic*, 13, 72 (1938). Fishmeal Putrefied Yielded Vitamin K.
- Quick, A. J., *J. Amer. Med. Assn.*, 110, 1658 (1938). Method Determining Prothrombin Level in Humans. *Science*, 92, 113 (1940). Thromboplastein Reagent for Examination of Prothrombin.
- Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 190, 501 (1935). Method for Determining Prothrombin.
- Smith, H. P., Warner, E. D., Brinkhous, K. M., and Seeger, W. H., *J. Exper. Med.*, 67, 911 (1938). Need for Bile Salts in Absorption from Vitamin K.
- Smith, H. P., Ziffren, S. E., Owen, C. A., and Doisy, E. A., *J. Amer. Med. Assn.*, 113, 380 (1939). Vitamin K and Biliary Jaundice.
- Snell, A. M., *J. Amer. Med. Assn.*, 112, 1457 (1939). Effect on Jaundice.
- Thayer, S. A., Binkley, S. B., McCorquodale, D. W., Doisy, E. A., Emmett, A. D., Brown, O. A., and Bird, O. D., *J. Am. Chem. Soc.*, 61, 2563 (1939). Vitamin K Value of 2-Methyl-1, 4-Naphthoquinone.

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Waddell, W. W., and Guerry, D., *J. Amer. Med. Assn.*, 112, 2259 (1939). Infant Hemorrhage.

Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 114, 667 (1936). Method for Determining Prothrombin; also First Report on Treatment of Bleeding in Cases of Obstructive Jaundice.

APPENDIX A

THE CHEMICAL NATURE OF THE VITAMINS

THE following pages present the formulas and chemical structures of those vitamins which have been identified.

Vitamin A

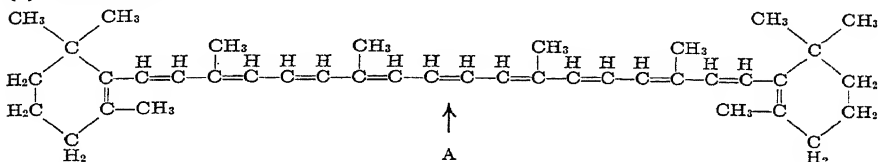
Vitamin A exists in three forms in nature: as the provitamin (carotene or cryptoxanthin), and in the active form, of which there are two types known as vitamins A_1 and A_2 . There are three forms of carotene, of which beta-carotene is the most active, yielding on hydrolysis two molecules of vitamin A_1 . Vitamin A_1 is the active form found in the livers of salt-water fish and vitamin A_2 in those of fresh-water fish.

In Figure 1a is shown the formula for beta-carotene and the manner in which it is split into two molecules of vitamin A. Note that this formula, if broken in two at point A, would give two identically constructed molecules. In the liver this is accomplished by hydrolytic cleavage at point A, *i.e.*, breaking by addition of water. The result is 2 molecules of vitamin A from one molecule of beta-carotene having the formula given in Fig. 1b.

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Figure 1. What Happens When Beta-Carotene Becomes Vitamin A.

(a) *Beta-Carotene*:



(b) *Vitamin A*:

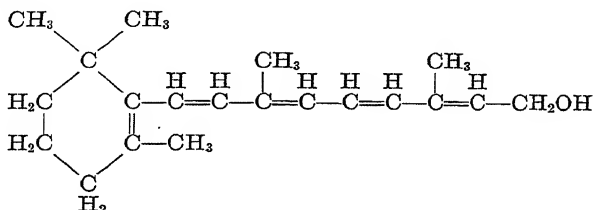
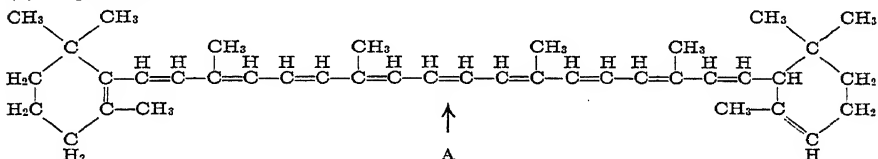
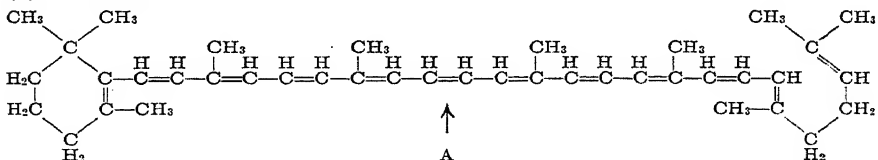


Figure 2. Formulas of Alpha- and Beta-Carotene and Cryptoxanthin.

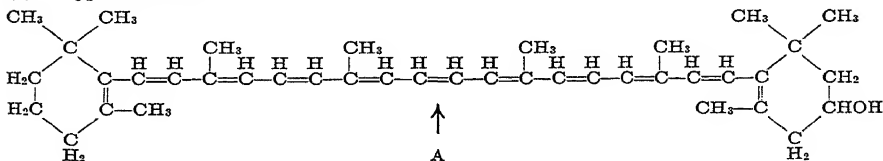
(a) *Alpha-Carotene*:



(b) *Gamma-carotene*:



(c) *Cryptoxanthin*:

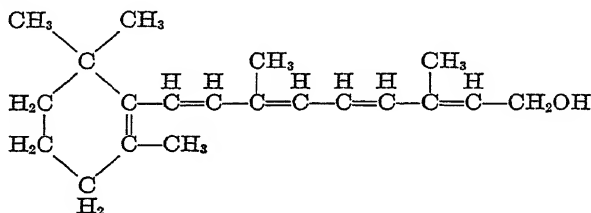


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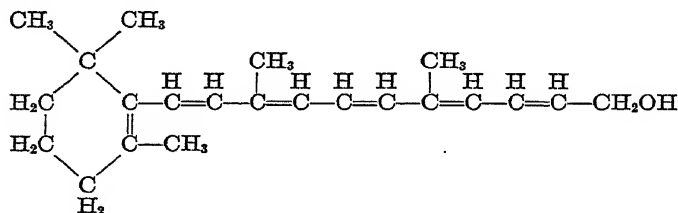
The formulas for the other forms of carotene and for the two forms of vitamin A are shown in Figures 2 and 3. The active vitamin A is an alcohol and may be converted into an ester. The carotenes and vitamin A are stable to heat, acids and alkalies, moderately sensitive to oxidation, labile to light, soluble in oils and fats, and nearly insoluble in water. The carotenes are bright yellow in color. The vitamins A are nearly colorless.

Figure 3. Two Forms of Active Vitamin A.

(a) *Vitamin A₁*:



(b) *Vitamin A₂*:



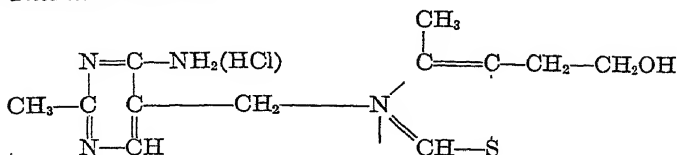
Vitamin B₁

Vitamin B₁ exists in animal tissues in two forms: as thiamine or as thiamine pyrophosphate, the latter being known as co-carboxylase. By oxidation these substances are converted into a blue fluorescent compound known as thiochrome.

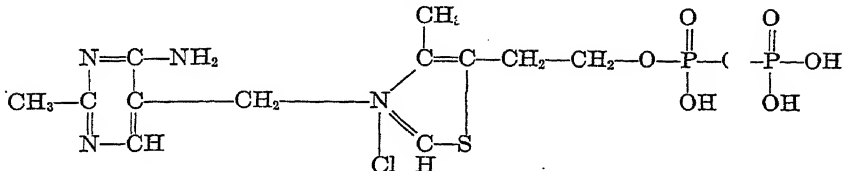
CHEMICAL NATURE OF VITAMINS

Figure 4. Thiamine and Related Products.

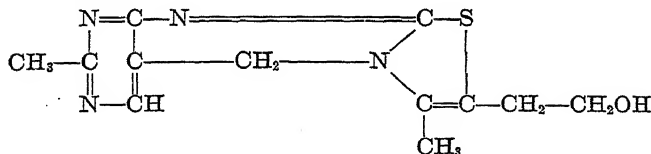
(a) *Thiamine Chloride*:



(b) *Co-carboxylase (Thiamine pyrophosphate):*



(c) *Thiochrome*:



The structure of these substances is shown in Figure 4. It will be noted that thiamine is actually a combination of a pyrimidine base with a sulfur ring compound known as thiazole. The synthetic product now available is the hydrochloride of thiamine. Thiamine is comparatively stable toward dry heat but is destroyed by continuous heat and by treatment with sulfides. It is soluble in water, and diffusible and insoluble in oils and fats. It is readily adsorbed on charcoal and fuller's earth.

Vitamin B₂ or Riboflavin

This vitamin, also known as vitamin G, consists of a ribose sugar attached to a colored compound called flavin.

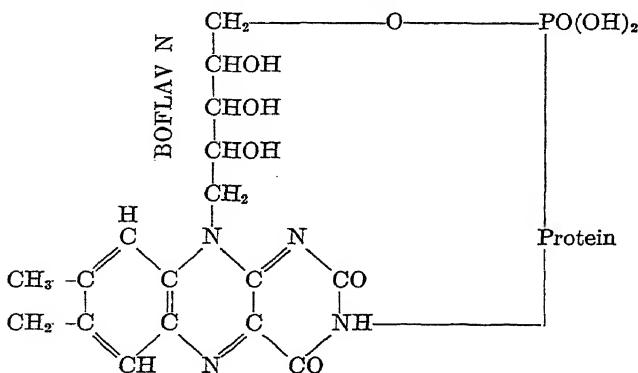
WHAT ARE THE VITAMINS?

In the early nomenclature of this vitamin the terms lacto-flavin, hepato-flavin, etc. were used to designate the source (milk, liver, etc.). With the discovery that all these compounds were alike and that all contained ribose sugar, the source names were dropped and the compound is today known as ribose flavin, or "riboflavin".

When riboflavin is phosphorylated it forms the prosthetic group in Warburg's yellow enzyme and acts as a hydrogen carrier. When protein is added to this prosthetic group an enzyme is produced whose specificity depends on the character of the protein, the protein acting as a specific adsorbent for certain substrates.

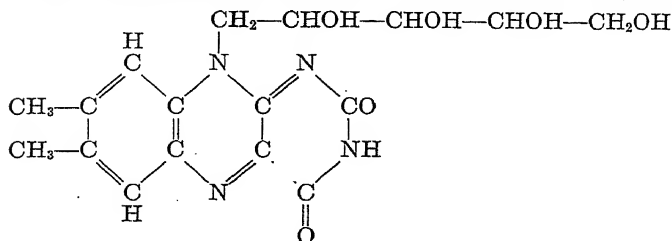
Riboflavin is soluble in water, stable to heat and fairly stable to oxidation, acids, and alkalis. It is rather sensitive to light. It is yellow-green in color and fluoresces in aqueous solution. The structure of riboflavin and Warburg's yellow ferment is shown in Figures 5 and 6.

Figure Theorell's Configuration of Warburg's Yellow Ferment or Respiratory Enzyme.



CHEMICAL NATURE OF VITAMINS

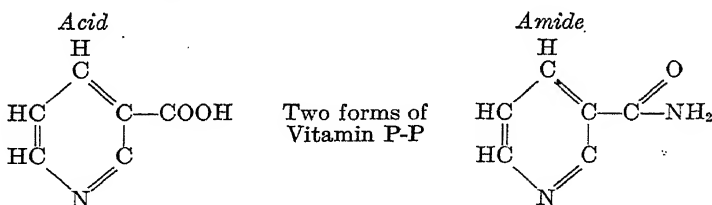
Figure 6. The Chemical Structure of Vitamin B₂ or G (Riboflavin).



Vitamin P-P (Goldberg's Anti-Pellagic Factor)

Elvehjem and associates showed that this factor was actually a form of nicotinic acid. Nicotinic acid is a 3-pyridine carboxylic acid. Its structure is shown in Figure 7 together with the structure of the amide, which is also active. It has also been shown to be a constituent of coenzymes 1 and 2 which act as hydrogen carriers in cellular respiration. The compound is soluble in hot water and alcohol.

Figure 7. Nicotinic Acid and Amide.



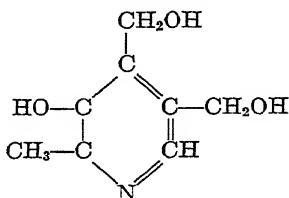
Vitamin B₆ (Pyridoxine)

This vitamin is also a pyridine compound. It was originally called adermin but the name has now been changed to pyridoxine as being better chemical description.

WHAT ARE THE VITAMINS?

Pyridoxine is water-soluble, stable to concentrated acid, heat and alkali. Structure is shown in Figure 8.

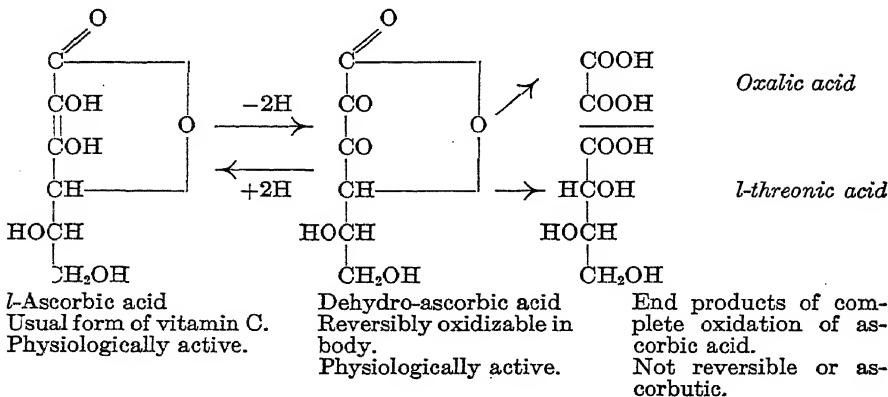
Figure 8. Vitamin B₆ (Pyridoxine).



Vitamin C (Ascorbic Acid)

This compound was originally known as hexuronic acid and is sometimes called cevitamic acid. The present accepted name is ascorbic acid. It exists in the active form in two conditions: as *l*-ascorbic acid, which is the more usual form, and as dehydro-ascorbic acid which is reversibly oxidizable in the body. On further oxidation dehydro-ascorbic acid is converted to oxalic and threonic acids which have no

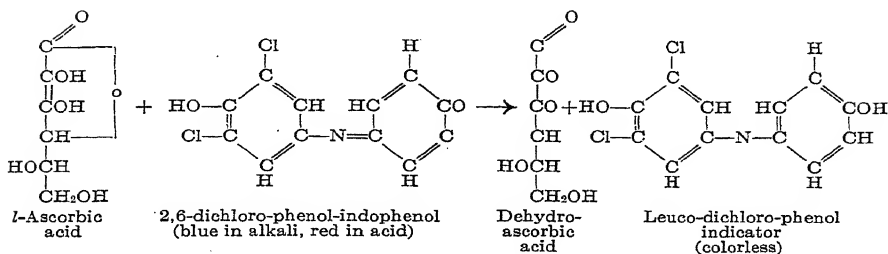
Figure 9.



CHEMICAL NATURE OF VITAMINS

Figure 10. Reaction of Ascorbic Acid with Indicator Dye.

[After Bessey, *J. Amer. Med. Assn.*, 111, 1290 (1938)]



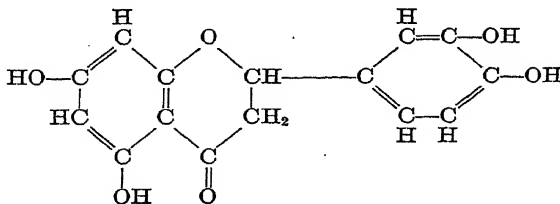
physiological activity. These forms are shown in Figure 9. *l*-Ascorbic acid is detectable by a dye which converts it to the dehydro- form and a colorless form of the indicator. Steps in this reaction are shown in Figure 10.

Ascorbic acid is insoluble in oils and readily soluble in water. It is particularly sensitive to alkalis and oxidation though fairly stable in weak, acid solutions. It is a strong reducing agent.

Vitamin P

It is debatable whether this compound is a true vitamin. Szent Györgyi claimed that it plays a role in prevention of hemorrhagic diathesis and that a combination of vitamin P

Figure 11. Structure of Eriodictiol (Vitamin P).



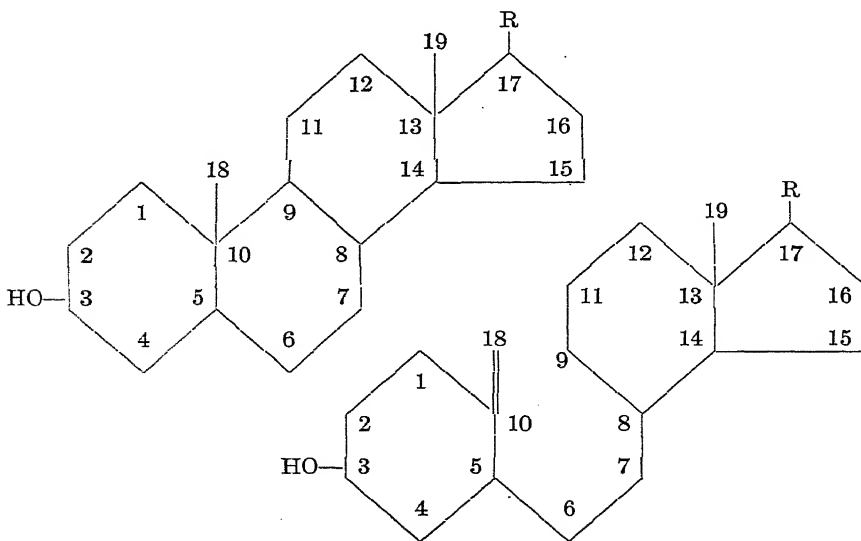
WHAT ARE THE VITAMINS?

and C in vegetable juices such as paprika is more effective than vitamin C alone. It appears to be a flavone compound whose structure is shown in Figure 11.

Vitamins D

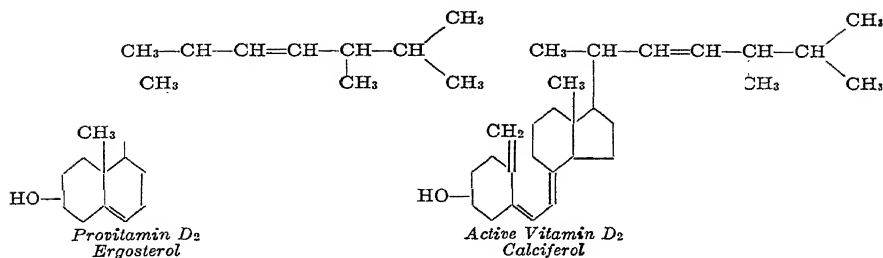
Two forms of vitamin D are now definitely recognized and known as D_2 and D_3 respectively. Vitamin D_2 is called calciferol. It is produced by activating ergosterol with ultra-violet light. Vitamin D_3 is a form of cholesterol known as 7-dehydro-cholesterol. Both of these are characterized by the presence of the sterol nucleus which is believed to undergo the changes shown in Figure 12 when activated by ultraviolet light or by other means.

Figure 12. The Sterol Nucleus of Vitamins D Before and After Activation.



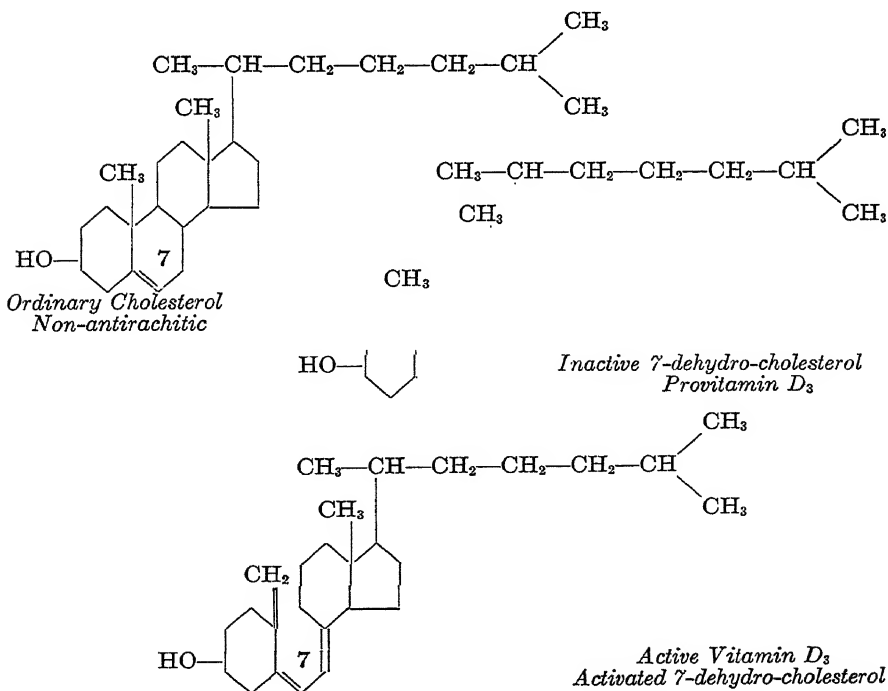
CHEMICAL NATURE OF VITAMINS

Figure 13



In Figures 13 and 14 is shown the chemical structure of vitamins D₂ and D₃. According to Bills, there are several

Figure 14. Relation of Cholesterol to Vitamin D₃ (7-dehydro-cholesterol).



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other forms of vitamin D, tested by ability to produce anti-rachitic effects. For description see Chapter Eleven.

Vitamins D are stable to heat, alkalies, acids, and oxidation. They are soluble in oils and fats and insoluble in water. Viosterol is the name given to a solution of calciferol in some inert oil and by U. S. Pharmacopeia methods must contain 10,000 I.U. of vitamin D₂ per gram.

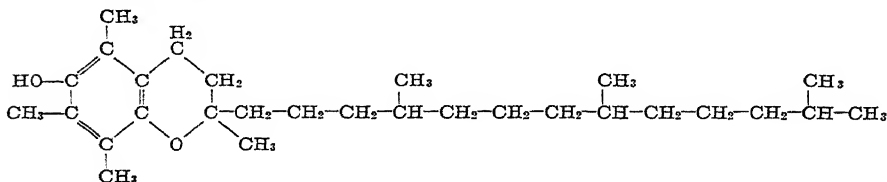
Vitamin E (Alpha-tocopherol)

The most active form of vitamin E appears to be the compound shown in Figure 15 as alpha-tocopherol. There is another form known as beta-tocopherol whose structure, according to Bergel, is also shown in Figure 15.

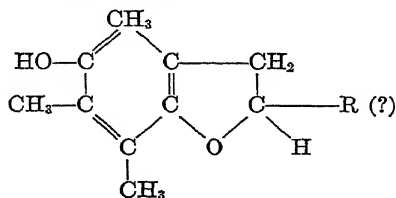
These vitamins are insoluble in water, soluble in oils and fats, stable to heat, alkalies and acids but destroyed by ferri-chloride in the presence of rancid fats.

Figure 15. Forms of Vitamin E.

(a) *Alpha-Tocopherol* according to Fernholz (1938); Chromane nucleus.



(b) *Beta-Tocopherol* according to Bergel (1938); Coumaran nucleus.



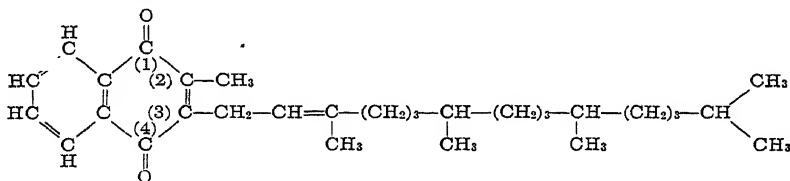
CHEMICAL NATURE OF VITAMINS

Vitamin K

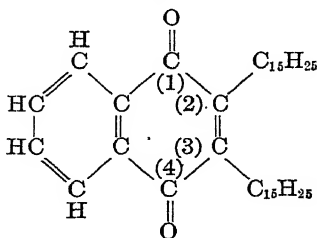
There are several forms of this vitamin. The two occurring in nature are known as K_1 and K_2 . All of them contain the 1,4-naphthoquinone nucleus and it has been shown that this nucleus with a methyl group in the 3-position is quite as active, if not more so, than the naturally occurring K_1 and K_2 . Several other substituted naphthoquinones have also been shown to have activity.

Figure 16. Forms of Natural Vitamin K and Their Relation to 1,4-Naphthoquinone.

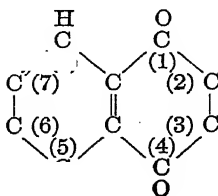
(a) *Natural Vitamin K_1* ; 2-methyl-3-phytyl-1,4-naphthoquinone:



(b) *Natural Vitamin K_2* ; 2,3-di-substituted naphthoquinone ($C_{41}H_{56}O_2$):



(c) *1,4-Naphthoquinone*:



WHAT ARE THE VITAMINS?

The vitamins K appear to be stable to light, heat and reducing agents but are destructible by alcoholic alkali oxidizing agents, strong acids and aluminum chloride. The naturally occurring forms are fat-soluble. Some of the synthetic forms are water-soluble. Structures are shown in Figure 16.

APPENDIX B

TABLE OF VITAMIN VALUES

SOIL conditions, methods of marketing, and methods of cooking may markedly affect vitamin values. It is therefore impossible to state definitely the vitamin content of any given foodstuff as it is served. The values given below, unless otherwise stated, are what appear to be the average we can expect from fresh, raw products of the type specified. The authority for the value given is indicated by the number in parentheses and the key to these numbers will be found in the bibliography at the end of the Table.

This table is limited to vitamins A, B₁, C, D, and G; values for other vitamins are limited today but such as are available have been given in the Chapters dealing with such vitamins and their functions.

The amounts of vitamins A, B₁, C, D, and G generally recommended to prevent deficiency may be stated as follows:

Type of Individual	Minimum daily need of Vitamins (Int. Units)				
	A	B ₁	C	D	G (micrograms)
Infant	1500	75	200	400	500
Child, 1 to 6	3000	125	400	400	?
Child, 6 to 12	3000	200	400	400	?
Over 12	4000	250	500	400	2000

WHAT ARE THE VITAMINS?

It will be noted that the values for vitamins A, B₁, C, and D are expressed in "International Units"; that for vitamin G in micrograms. The meaning of these units in actual amount of vitamin substance is as follows:

Definition of Units

One Int. Unit of vitamin A is the amount necessary to produce the physiological effect of 0.6 microgram (0.0006 milligram) of pure beta-carotene.

One Int. Unit of vitamin B₁ is the amount necessary to produce the physiological effect of 3.0 micrograms (0.003 milligram) of pure crystalline thiamine chloride.

One Int. Unit of vitamin C is the amount necessary to produce the physiological effect of 0.05 milligram of pure crystalline ascorbic acid.

One Int. Unit of vitamin D is the amount necessary to produce the physiological effect of 0.025 micrograms (0.000025 milligram) of pure calciferol.

The value of vitamin G was formerly expressed and is still given on some labels in "Sherman-Bourquin" units but that unit is now defined as equivalent to 3 micrograms of pure riboflavin and in our Table we give vitamin G values in micrograms of riboflavin.

The values given on pages 220-230 are the approximate amounts present in 100 grams of foodstuff. Since 100 grams equals 3.5 oz., the value per ounce is obtainable by dividing by this factor (3.5).

TABLE OF VITAMIN VALUES

Bibliography

- ¹ Munsell, H., *Milbank Memorial Fund Quarterly*, 18, 311 (1940).
- ² Boller, A., Personal communication from records of Nat'l Livestock and Meat Board.
- ³ Hodson, A. Z., *Food Research Journal*, 5, 395 (1940).
- ⁴ Booher, L. E. and Hartzler, E. R., *Techn. Bull.* 707, U. S. Dept. Agric., December 1939.
- ⁵ Eddy, W. H., and Morris, S. G., *Journal Pediatrics*, 4, 208 (1934).
- ⁶ Morgan, A. F., Kimmel, L., and Davison, H. G., *Food Research Journal*, 4, 145 (1939).
- ⁷ Bureau of Nutrition, Borden Company.
- ⁸ Quinn, E. J., and Brabec, L. B., *J. Home Econ.*, 22, 123 (1930).
- ⁹ Fitzgerald, G. A., and Fellers, C. R., *Food Research Journal*, 3, 109 (1938).
- ¹⁰ Sherman, H. C., "Chemistry of Food and Nutrition," 5th Ed., Macmillan, 1937.
- ¹¹ Guerrant, M. B., Dutcher, R. A., Tabor, F. S., and Rasmussen, R. J., *J. Nutrition*, 11, 383 (1936).
- ¹² Zimmerman, W. L., Tressler, D. K., and Maynard, L. A., *Food Research Journal*, 5, 93 (1940).
- ¹³ Stimson, C. R., Tressler, D. K., and Maynard, L. A., *Food Research Journal*, 4, 475 (1939).
- ¹⁴ Daniell, E. P., and Munsell, H. K., *Misc. Public.* 275, U. S. Dept. Agric. 1937.
- ¹⁵ Floyd, W. W., and Fraps, G. S., *Food Research Journal*, 4, 87 (1939).
- ¹⁶ Arnold, A., and Elvehjem, C. A., *Food Research Journal*, 4, 547 (1939).
- ¹⁷ Mickelson, O., Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, 17, 269 (1939).
- ¹⁸ Williams, R. R., and Spies, T. D., "Vitamin B₁₂," Macmillan, 1938.
- ¹⁹ Harris, P. L., and Poland, G., *Food Research Journal*, 2, 311 (1937).
- ²⁰ Gunderson, F. L., *Personal communication*.
- ²¹ Darby, W. J., and Day, P. L., *J. Nutrition*, 16, 209 (1938).
- ²² Day, P. L., and Darby, W. J., *Food Research Journal*, 1, 349 (1936).
- ²³ Newton, C. L., *Bull.* 167, Georgia Exper. Station, 1931.
- ²⁴ Morgan, A. F., Nobles, H. L., Wiens, A., Marsh, G. L., and Winkler, A. J., *Food Research Journal*, 4, 217 (1939).
- ²⁵ Bessey, O. A., *J. Am. Med. Assn.*, 111, 1290 (1938).
- ²⁶ Pederson, C. S., Mack, G. L., and Athawes, W. L., *Food Research Journal*, 4, 31 (1939).

WHAT ARE THE VITAMINS?

Vitamin Content

Foodstuff	International Units of Vitamins			
	per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
<i>I. Meats and Meat Substitutes</i>				
Bacon	25 ¹	33 ¹⁸		0 ¹
Beef, ave., lean	56 ²	25-38 ²		
Brain, beef	54 ²	56 ²		
Cheese, Cheddar	2000 ¹	14 ⁴		
Cheese, cottage	500 ¹			
Cheese, cream	2100 ¹			
Chicken, white meat	0 ¹	25-48 ²		
Chicken, dark meat	0 ¹	59-77 ²		
Chicken, ave.	0 ¹	43 ²		
Egg, whole hen's	1000 ¹	50 ¹⁸	0 ²⁵	
Egg, white	0 ¹	0 ⁴	0 ²⁵	
Egg, yolk	2800 ¹	118 ⁴	0 ²⁵	
Fish, ave.		13 ¹⁸		
Fish, cod	5 ¹	30 ¹		
Fish, haddock	5 ¹	5 ¹		
Fish, halibut		28 ⁴		
Fish, salmon	30-750 ¹	tr. ¹		800 ¹
Fish, sardines		10 ¹		
Ham, fresh	(?)	303-510 ²		
Ham, smoked	(?)	358-476 ²		
Heart, beef	tr.	225 ²		
Heart, pork		174 ²		
Heart, sheep	tr.	150 ²		
Kidney, beef	1000 ¹	105 ²		
Kidney, lamb	1000 ¹	150-190 ²		
Kidney, veal	1000 ¹	60 ²		
Lamb, ave., lean	(?)	60-111 ²		
Liver, beef	9000 ¹	89-129 ²	750 ²	45 ²
Liver, calf	5475 ²	45 ²	650 ²	15 ²
Liver, chicken		75 ²	450 ²	50 ²
Liver, lamb	5475 ²	100-138 ²	750 ²	20 ²
Liver, pork	6000 ²	156 ²	525 ²	45 ²
Lung, beef		70 ¹⁶		
Pork, ave.	tr.	303-510 ²		
Tongue, beef		100 ¹⁷		
Veal, ave., lean	(?)	40-112 ¹⁷		
<i>II. Dairy Products</i>				
Butter, ave.	2400 ¹	35 ⁷	0 ²⁵	80 ¹
Cheese, Cheddar	2000 ¹	14 ⁴		
Cheese, cottage	500 ¹			

TABLE OF VITAMIN VALUES

of Foodstuffs

Micrograms of Vitamin G (riboflavin) per 100 g.	Average Portion or Serving	Oz.	Grams	per Serving				Micro- grams Vita- min G (ribo- flavin)
				(Int. Units of Vitamins)				
				A	B ₁	C	D	
30 ²¹	4 strips, 8½" long	2.6	80	20	26	24
375 ²	½ lb.	8	230	129	87	860
251 ¹	½ lb.	8	230	124	129	577
750 ¹⁰	1½" x 1½" x 1¼"	1	25	500	4	188
	¼ cup	2	55	275
138 ²²	¼ cup	2	55	1155	759
74-82 ²	½ lb.	8	230	0	85	179
258 ²	½ lb.	8	230	0	156	593
138 ²	½ lb.	8	230	0	100	759
330 ¹	One	2	50	500	25	165
300 ¹	One	1.25	35	0	0	105
345 ¹	One	0.5	15	620	18	518
	½ lb.	8	230	12	30
	½ lb.	8	230	12	70
	½ lb.	8	230	12	12
	½ lb.	8	230	..	64
225 ¹	½ lb.	8	230	..	tr.	..	1840	518
	½ lb.	8	230	..	23
195-300 ²	½ lb.	8	230	0	900	500
156-300 ²	½ lb.	8	230	0	960	959
767-900 ²	¼ lb.	4	115	tr.	260	958
115 ²	¼ lb.	4	115	0	200	1282
834-3000 ²	¼ lb.	4	115	tr.	173	2205
1872-2400 ²	¼ lb.	4	115	1150	163	2456
1980 ²	¼ lb.	4	115	1150	196	2272
2400-2700 ²	¼ lb.	4	115	1150	70	2933
331 ²	½ lb.	8	230	..	196	761
3000-3700 ²	½ lb.	8	230	20700	250	1725	104	7705
2700-3900 ²	½ lb.	8	230	12592.	52	1495	35	7590
	½ lb.	8	230	..	173	1035	115	..
2610-2990 ²	½ lb.	8	230	12592	274	1725	46	6440
2625-2900 ²	½ lb.	8	230	13800	360	1208	104	5352
	¼ lb.	4	115	..	80
287 ²	½ lb.	8	230	tr.	936	660
	5 slices	2.5	75	..	75
345 ²	½ lb.	8	230	..	168	794
0 ¹	2 tbs.	1	30	720	11	..	24	0
750 ¹⁰	1 cube	1	25	500	4	188
	1½" x 1½" x 1¼"							
	¼ cup	2	55	275

WHAT ARE THE VITAMINS?

Vitamin Content

Foodstuff	INTERNATIONAL UNITS OF VITAMINS per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
Cheese, cream	2100 ¹			
Cream, 20%	680 ¹	10 ⁷		
Eggs, whole hen's	1000 ¹	50 ¹⁸	0 ²⁵	
Eggs, white	0 ¹	0 ⁴	0 ²⁵	
Eggs, yolk	2800 ¹	118 ⁴	0 ²⁵	
Margarines, fortified	1800	0		
Milk, whole, fluid	110 ¹	16 ⁴	25-40 ²⁵	2
Milk, whole, evaporated	670 ⁷	24 ⁴		
Milk, whole, dried	875 ¹	105 ⁴	100 ²⁵	16
Milk, skim, fluid	2 ¹	14 ⁴		
Milk, human	110 ⁸	10-12 ⁵	120 ²⁵	
<i>III. Shellfish</i>				
Clams	14 ¹	7 ¹		
Crabmeat				
Lobster				
Oysters	140 ¹	75 ¹⁴	5 ¹	
<i>IV. Nuts</i>				
Almonds	75 ¹	75 ¹	0 ²⁵	
Chestnuts		57 ¹⁸	0 ²⁵	
Coconut		20 ¹		
Filberts		206 ¹⁸	0 ²⁵	
Hazel	100 ¹	220 ¹	0 ²⁵	
Peanuts		250-300	0 ²⁵	
Pecans	400 ¹	350 ⁴	0 ²⁵	
Walnuts, black	130 ¹	114 ⁴	0 ²⁵	
Walnuts, English	100 ¹	150 ⁴	0 ²⁵	
<i>V. Cereals</i>				
Barley	0 ¹	120 ¹	0	
Bran, wheat	140 ¹	100-360 ¹⁰		
Bread, rye	tr.	70 ¹		
Bread, white, wheat	tr.	75 ¹⁸		
Bread, whole wheat	tr.	1133 ¹⁸		
Buckwheat		66 ¹⁸		
Cornmeal, white	0	101 ⁴		
Cornmeal, yellow	500 ¹	78 ⁴		
Farina	0 ¹	24-43 ²⁰		
Flour, graham		110-150 ¹⁰		

TABLE OF VITAMIN VALUES

of Foodstuffs

Micrograms of Vitamin G (riboflavin) per 100 g.	Average Portion or Serving	Oz.	Grams	per Serving				Micro- grams Vita- min G (ribo- flavin)
				(Int. Units of Vitamins)				
				A	B ₁	C	D	
138 ²²	Cube 2" x 1" x 1½"	1	30	1155	759
	1 cup	8	230	1564	23
330 ¹	One	2	50	500	25	165
300 ¹	One	1.25	35	0	0	105
345 ¹	One	0.5	15	620	18	518
	2 tbs.	1	30	540	54	..
225 ⁷	1 cup	8	230	253	37	74	4	518
303 ⁷	½ cup	4	165	253	57	..	4	518
1500 ¹	⅓ cup	1	30	253	32	30	4	518
180 ¹	1 cup	8	230	5	42	..	?	314
750 ¹	1 cup	8	230	253	25	276	?	1725
	½ cup	3.5	100	14	7
	⅓ cup	2	65	910	49	3
600 ¹	20	1	30	23	23	0	..	200
	8	2	50	..	28	0
	¼ cup	2	60	..	12
	¼ cup	1.25	35	..	72	0
	¼ cup	1.25	35	..	132	0
60 ¹⁰	⅓ cup	2	60	..	165	0	..	36
300 ¹	6	1	25	100	88	0	..	75
	6	1.25	35	46	40	0
	6	1.25	35	35	52	0
0 ¹	3 tbs.	1	30
	½ oz.	0.5	15
	1 slice	1	25	tr.	17
0 ¹	1 slice	1	25	tr.	4	0
120 ¹⁰	1 slice	1	25	tr.	33	30
	¼ cup	2	50	..	33
99 ¹⁰	¼ cup	2	50	..	50
	¼ cup	2	50	250	39	50
	¼ cup	2	50	..	17
	⅔ cup	3.5	100	..	130

WHAT ARE THE VITAMINS?

Vitamin Content

Foodstuff	International Units of vitamins			
	per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
Flour, rye	o	56 ¹⁸		
Flour, patent wheat	o	17 ⁴		
Flour, patent plus germ		43 ⁴		
Flour, straight milled wheat		29 ⁴		
Flour, whole wheat		160-190 ²		
Hominy, white	o ¹⁴	66 ¹⁸		
Hominy, yellow	60o ¹⁴	66 ¹⁸		
Oats	tr. ¹	210-288 ²⁰		
Oatmeal	tr. ¹	190-270 ²⁰		
Pastes, macaroni	tr. ¹	16 ¹⁸		
Pastes, sago	o ¹	3 ¹⁸		
Pastes, tapioca	o ¹	3 ¹⁸		
Rice, brown	tr. ¹	159-175 ²⁰		
Rice, white	tr. ¹	10 ¹⁸		
Rice polish		666-1250 ¹⁸		
Rye	o ¹	131-156 ⁴		
Wheat	tr. ¹	118-175 ²⁰		
Wheat germ		600-1333 ¹⁸		

VI. Fruits

Apples, ave.	75 ¹	8-15 ¹	30-40o ¹
Apricots, fresh	400o ¹	10 ¹	20 ²⁵
Apricots, dried	500o ¹	30 ¹	160 ²⁵
Avocados	100 ¹	34 ⁴	40o ¹
Bananas	30o ¹	14-18 ¹⁹	220 ¹⁹
Blackberries	15o ¹	8 ⁴	60 ²⁵
Blueberries	100 ¹	16 ⁴	90-120 ¹
Cantaloupe	30o ¹	20 ¹	60o ¹
Cherries	20o ¹	17 ⁴	160 ²⁵
Cranberries	20 ¹		200 ²⁵
Currants, red		15 ¹	300 ²⁵
Dates, dried	15o ¹	25 ¹	o ²⁵
Figs, fresh	5o ¹	25 ¹	36 ¹⁵
Figs, dried	6o ¹	22 ¹	o ¹
Grapefruit	o ⁹	24 ⁴	85o ¹
Grapefruit juice	o ⁹	25 ¹	80o ²⁵
Grapes, ave.	tr. ¹	15 ¹	6o ¹
Gooseberries			50o ¹
Lemons	tr. ¹	10 ¹	800-90o ¹
Lemon juice	o ¹	10 ¹	1200 ²⁵
Lime juice			75o ¹⁵
Mango	150o ¹	30 ¹	60o ¹
Muskmelon		19 ⁴	244 ¹⁵

TABLE OF VITAMIN VALUES

of Foodstuffs

Micrograms of Vitamin G (riboflavin) per 100 g.	Average Portion or Serving	Oz.	Grams	per Serving				Micro- grams Vita- min G (ribo- flavin)
				(Int. Units of Vitamins)				
				A	B ₁	C	D	
O ¹	$\frac{2}{3}$ cup	3.5	100	..	58
	$\frac{2}{3}$ cup	3.5	100	..	17	0
	$\frac{2}{3}$ cup	3.5	100	..	43
	$\frac{2}{3}$ cup	3.5	100	..	29
	$\frac{2}{3}$ cup	3.5	100	..	175
105 ¹	$\frac{1}{4}$ cup	2	50	0	33
	$\frac{1}{4}$ cup	2	50	300	33
	$\frac{1}{4}$ cup	1.6	40	tr.	200	42
	$\frac{1}{4}$ cup	1.6	40	tr.	92	42
	$\frac{1}{2}$ cup	2.5	75	tr.	8
105 ¹	$\frac{1}{4}$ cup	1.6	40	0	1
	$\frac{1}{4}$ cup	1.6	40	0	1
	1 tsp.	.6	20	tr.	33	30
	1 tsp.	.6	20	tr.	2	tr.

105 ¹	$\frac{1}{4}$ cup	2	50	0	143	53
105 ¹	$\frac{1}{4}$ cup	2	50	tr.	93	53
450-1212 ¹	1 tsp.	.6	20	..	193	166
73 ³	One, 2 $\frac{1}{2}$ " diam.	4.6	130	98	16	279	..	95
51 ¹	2	2	50	2000	5	10	..	25
105 ¹	6 halves	1	25	1250	7	40	..	23
90 ¹	$\frac{1}{2}$	3	85	85	29	340	..	77
84 ³	One	4.5	125	375	20	275	..	71
73 ³	$\frac{2}{3}$ cup	2.8	75	111	6	45
	$\frac{2}{3}$ cup	3.5	100	100	16	105
	$\frac{1}{2}$	7	200	600	40	1200	..	146
	15	3	85	170	14	136
	1 cup	4.6	130	26	..	260	..	0
45 ¹	$\frac{1}{2}$ cup	2	50	..	7	150
	2	$\frac{1}{2}$	13	20	3	0	..	6
	2	2	50	25	13	18	..	27
	6 halves	1	25	15	5	0	..	18
	$\frac{1}{2}$	3.5	100	0	24	850	..	tr.
45 ¹	$\frac{1}{2}$ cup	4.5	120	0	29	960	..	tr.
75 ¹	20	3.5	100	tr.	15	60	..	65
39-92 ³	$\frac{1}{2}$ cup	4.5	100	500
	One	3.5	100	tr.	10	850
	1 tsp.	$\frac{1}{2}$	15	0	2	180	..	tr.
	$\frac{1}{2}$ cup	3.5	100	750
	One	3.5	100	1500	30	600	..	60
60 ¹	$\frac{1}{2}$	7	200	..	38	488

WHAT ARE THE VITAMINS?

Vitamin Content

Foodstuff	International Units of vitamins per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
Olives, green	190 ¹		0 ¹	
Olives, ripe	125 ¹	2 ¹	0 ¹	
Oranges	65 ⁹	26 ⁴	760-900 ⁹	
Orange juice	45-350 ¹	30 ¹	450-1200 ¹	
Papaya	2500	25 ¹	800 ²⁵	
Peaches, yellow	1000 ¹	10 ¹	140-263 ²⁵	
Peaches, yellow, dried	3000 ¹	17 ¹	500 ²⁵	
Peaches, white	5 ¹	8 ⁴	200 ²⁵	
Pears, ave.	10 ¹	8 ⁴	48-60 ²⁵	
Persimmons			820 ¹⁵	
Pineapple	90 ¹	30 ⁴	500 ²⁵	
Pineapple juice	147 ¹¹	25 ¹¹	140 ¹¹	
Plums		16 ⁴	40-116 ²⁵	
Pomegranate juice			120 ¹⁵	
Prunes, dried	2500 ¹	60 ⁴	0 ²⁵	
Quince			100 ²⁵	
Raspberry	520 ⁹	8 ⁴	300 ²⁵	
Strawberry	740 ⁹	8 ⁴	1000 ²⁵	
Tangerine		30 ¹	700 ¹⁵	
Watermelon	125 ¹⁰	20 ¹	120-300 ²⁵	
Youngberry	450 ⁹			
<i>VII. Vegetables</i>				
Artichokes	200 ¹	60 ¹	175 ¹	
Asparagus, green	700 ¹	70 ¹	348-800 ²⁵	
Asparagus, white	0-50 ¹	59 ¹	650 ¹	
Beans, green, snap	1000 ¹	24 ¹	300 ¹	
Beans, lima, green	500 ¹	114 ⁴	600 ¹	
Beans, soy, fresh	200 ¹	175 ¹	800 ¹	
Beans, wax	0 ¹	29 ⁴	300 ¹	
Beans, kidney, dry	(?)	150 ¹	0 ¹	
Beans, lima, dry	100 ¹	170 ⁴	0 ¹	
Beans, navy, dry	0 ¹	128 ⁴	0 ²⁵	
Beans, soy, dry	100 ¹	485 ⁴	0 ¹	
Broccoli	9000 ¹	33 ⁴	1400-2600 ¹	
Brussels sprouts	200 ¹	57 ⁴	1000 ²⁵	
Cabbage, head	0-100 ¹	27 ⁴	60-128 ²⁵	
Carrot, fresh	2100 ¹	24 ⁴	60-128 ²⁵	
Cauliflower	30 ¹	56 ⁴	1500 ¹	
Celery, bleached	10 ¹	12 ⁴	100 ²⁵	
Chard	9000 ¹		100-460 ²⁵	
Collards	7000 ¹	67 ⁴	200-1200 ²⁵	
Corn, sweet, white	0-50 ¹	40 ⁴	200 ¹	
Corn, sweet, yellow	500 ¹	50 ⁴	163-200 ²⁵	

TABLE OF VITAMIN VALUES

of Foodstuffs

Micrograms of Vitamin G (riboflavin) per 100 g.	Average Portion or Serving	Oz.	Grams	-per Serving-				Micro- grams Vita- min G (ribo- flavin)
				(Int. Units of — Vitamins) —				
				A	B ₁	C	D	
	5	1	25	48	..	0		..
0 ¹	5	.6	20	25	0.4	0		0
15 ¹	One	3.5	100	65	26	800		18
15 ¹	½ cup	4.5	120	234	36	990		15
180 ¹	½	3.5	100	2500	25	800		180
60 ¹	2 halves	5	140	1400	14	281		84
	3 halves	2	50	1500	9	250		
	2 halves	5.3	150	7	12	300		
76 ³	One	5.3	150	15	12	81		114
	One	2	50	..		410		..
36 ¹	1 cup	5.3	150	135	45	750		54
21 ¹	½ cup	4.5	120	176	30	168		25
45 ¹	2	2	50	..		78		27
	One	10	310	3610		..
1176 ¹	8	3.5	100	2500	60	0		1176
	One	8.6	240	240		
	¾ cup	2.8	75	390	6	225		
tr. ¹	¾ cup	3.5	100	740	8	1000		tr.
30 ¹	One	3.5	100	..	30	700		30
30 ¹	2 slices, 2" diam.	3.5	100	125	20	210		30
14 ¹	¾ cup	2.8	75	336				11
	One, 3" diam.	5.3	150	300	90	262		
129 ³	6, 6" stalks	2.8	75	525	53	432	..	96
	6, 6" stalks	2.8	75	18	45	486
93 ³	¾ cup	2.8	75	750	18	225	..	39
900 ¹	½ cup	2.8	75	375	84	450	..	675
300 ¹	½ cup	2.8	75	150	132	600	..	225
120 ¹	¾ cup	4.5	130	0	38	390	..	156
	1½ cup	2.8	75	0	111	0
900 ¹	1½ cup	2.8	75	75	126	0	..	675
1200 ¹	1½ cup	2.8	75	0	96	0	..	900
900 ¹	½ cup	3.5	100	100	485	0	..	900
210 ³	1 cup	4.2	120	10800	40	2400	..	252
90-180 ¹	1 cup	3.5	100	200	57	1000	..	135
44-66 ³	1 cup	4.5	130	65	35	845	..	72
67 ³	¾ cup	4.2	120	2520	29	113	..	80
127 ³	1¼ cup	4.4	125	37	70	1875	..	159
35 ¹	2, 7" stalks	1.4	40	4	5	40	..	14
138 ¹	1 cup	3.5	100	9000	..	280	..	138
300 ¹	¾ cup	3.5	100	7000	67	700	..	300
	1 ear, 8"	3.5	100	25	40	200
121 ¹	1 ear, 8"	3.5	100	500	50	180	..	121

WHAT ARE THE VITAMINS?

Vitamin Content

Foodstuff	International Units of Vitamins			
	per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
Cucumber	20 ¹	15 ¹	40 ²⁵	
Egg plant	35 ¹	15 ¹	42-100 ²⁵	
Endive	15000 ¹	33 ⁴	400 ¹	
Escarole	15000 ¹	28 ¹	400 ¹	
Greens, beet			700 ²⁵	
Greens, dandelion	12000 ¹		800 ²⁵	
Greens, mustard		45 ⁴	1200 ²⁵	
Greens, turnip	10000 ¹	46 ⁴	600 ¹	
Kale	20000 ¹	63 ⁴	2500 ¹	
Kohlrabi		20 ¹	1200 ¹	
Leeks		50 ¹	300 ²⁵	
Lentils, dry	tr. ¹	170 ¹	0 ¹	
Lettuce, green	4000 ¹	25 ¹	200 ²⁵	
Lettuce, head	100 ¹	29 ⁴	100 ²⁵	
Mushrooms	0 ¹	30 ¹	0 ¹	
Mustard		3 ¹⁸	3300 ¹⁵	
Okra	400 ¹	42 ⁴	200 ²⁵	
Onions	0 ¹	10 ⁴	200 ²⁵	
Parsley	30000 ¹		2000 ¹	
Parsnips	tr. ¹	40 ¹	450 ¹	
Peas, green	1000 ¹	140 ¹	300 ²⁵	
Peas, dried cowpeas	50 ¹	312 ⁴	0 ¹	
Peppers, red	5000 ¹	10 ¹	4600 ²⁵	
Peppers, green	5000 ¹	10 ¹	2709 ²⁵	
Plantains			14 ¹	
Potatoes, sweet	3500 ¹	31 ⁴	160-406 ²⁵	
Potatoes, white	30 ¹	62 ⁴	140-300 ²⁵	
Pumpkin	2500 ¹	15 ¹	100 ²⁵	
Radish	tr. ¹	20 ¹	240 ²⁵	
Rhubarb	tr. ¹	8 ⁴	300 ²⁵	
Romaine	800 ¹		250 ¹	
Rutabaga, white	0 ¹	15 ¹	600 ²⁵	
Rutabaga, yellow	25 ¹	25 ⁴	400 ¹	
Sauerkraut	25 ¹	10 ⁴	0-100 ²⁵	
Sauerkraut juice		35 ⁴	180-206 ²⁵	
Shallots			300 ¹⁵	
Spinach	25000 ¹	35 ⁴	1500 ¹	
Squash, Hubbard	4000 ¹	16 ⁴	60 ²⁵	
Squash, summer	1000 ¹	14 ⁴		
Tomato	1000 ¹	20-26 ⁴	360-400 ²⁵	
Tomato juice	1000 ¹	20-26 ⁴	250-600 ¹	
Turnip, white	0 ¹	20 ⁴	600 ²⁵	
Turnip, yellow	20 ¹	12 ¹	600 ²⁵	
Watercress	4000 ¹	40 ¹	1000 ²⁵	

TABLE OF VITAMIN VALUES

of Foodstuffs

Micrograms of Vitamin G (riboflavin) per 100 g.	Average Portion or Serving	Oz.	Grams	per serving				Micro- grams Vita- min G (ribo- flavin)
				(Int. Units of Vitamins)				
				A	B ₁	C	D	
24 ¹	One	2.8	75	15	12	30	..	18
30 ¹	2 slices	8.8	250	87	37	177	..	75
120 ¹	1/2 head	1.6	45	6750	149	180	..	54
120 ¹	1/2 head	1.6	45	6750	13	180	..	54
82 ³	1 cup	3.5	100	700	..	82
225 ¹⁰	1/2 cup	1.7	50	6000	..	400	..	113
450 ¹⁰	1 cup	3.5	100	..	45	1200	..	450
360 ¹	1 cup	3.5	100	10000	46	600	..	360
600 ¹	1 3/4 cup	4.3	175	35000	111	4375	..	1050
75 ¹⁴	1/2 cup	3.5	100	..	20	1200	..	75
	1/2 cup	2	55
315 ¹	2 tbs.	1	25	tr	42	0	..	80
71 ³	2 leaves	1.7	50	2000	13	100	..	35
48 ³	1/2 head	1.7	50	50	15	50	..	24
0 ¹	1/2 cup	1.7	50	0	15	0	..	0

	7, 2 1/2" pods	1.7	50	200	21	100
123 ³	One, 2" diam.	1.7	50	0	5	100	..	66
	1 tsp.	0.03	1	300	..	20
	3/4 cup	4.2	120	tr.	48	540
130-150 ³	1/2 cup	2.8	75	750	105	225	..	105
300 ¹	..	3.5	100	50	312	0	..	300
138 ³	3" piece	1	25	1200	2	1150	..	34
138 ³	3" piece	1	25	1200	2	677	..	34
	One	3.5	100	14
68-70 ³	One	5.3	150	5250	46	425	..	105
45-55 ³	One	3.5	100	30	62	220	..	50
45 ¹	3/4 cup	4.2	120	3000	18	120	..	54
30 ¹	6 med.	1.7	50	tr.	10	120	..	15
	1 cup	3.2	90	tr.	7	270
46 ¹	5 leaves	1.7	50	400	..	125	..	23
	3/4 cup	4.2	120	0	18	720
	3/4 cup	4.2	120	30	30	480
	1/2 cup	2.8	80	20	8	40
	1/2 cup	4.2	120	..	42	463
	4	0.3	12	36
160 ³	1 1/2 cup	2.8	75	18750	27	1125	..	120
46 ¹	1 3/4 cup	8.8	250	10000	40	150	..	115
52 ¹	1 3/4 cup	8.8	250	2500	35	130
52 ³	One	4.4	125	1250	28	475	..	65
36-174 ¹	1/2 cup	4.2	120	1200	28	570	..	126
42 ³	3/4 cup	4.2	120	0	24	720	..	50
36 ¹	3/4 cup	4.2	120	24	14	720	..	44
270 ¹	1/2 cup	0.6	20	800	8	200	..	54

WHAT ARE THE VITAMINS?

Vitamin Content of Foodstuffs

Foodstuff	International Units of vitamins per 100 g. (3.5 oz.) of fresh, raw material			
	A	B ₁	C	D
<i>VIII. Miscellaneous</i>				
Lard, hog	7 ¹⁵	33 ¹⁸		
Molasses	0 ¹	tr. ⁴		
Oils, corn	0 ¹	0 ¹		
Oils, cottonseed	0 ¹	0 ¹		
Oils, coconut	0 ¹	0 ¹		
Oils, olive	tr. ¹	0 ¹		
Oils, peanut	0 ¹	0 ¹		
Oils, U.S.P. cod liver	85000 ¹			85000 ¹
Oils, mackerel liver	16000-211000 ⁶			2700-7400 ⁶
Oils, salmon liver	5800-57000 ⁶			475-570 ⁶
Oils, salmon body	525 ⁶			58-142 ⁶
Oils, sardine body	300 ⁶			95 ⁶
Oils, white sea bass liver	45000-180000 ⁶			1400-3500 ⁶
Oils, shark liver	5500-120000 ⁶			< 18 ⁶
Oils, blue fin tuna liver	78000 ⁶			35000-54000 ⁶
Oils, halibut liver	100000 ⁶			
Wines, ave.			3-4 ²⁴	
Yeast, bakers		233 ¹⁸		
Yeast, brewers		666 ¹⁸		

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